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(54) Title: NOVEL SERPENTINE TRANSMEMBRANE ANTIGENS EXPRESSED IN HUMAN CANCERS AND USES THEREOF

## (57) Abstract

Described is a novel family of cell surface serpentine transmembrane antigens. Two of the proteins in this family are exclusively or predominantly expressed in the prostate, as well as in prostate cancer, and thus members of this family have been termed "STRAP" (Serpentine TRansmembrane Antigens of the Prostate). Four particular human STRAPs are described and characterized herein. The human STRAPs exhibit a high degree of structural conservation among them but show no significant structural homology to any known human proteins. The prototype member of the STRAP family, STRAP-1, appears to be a type IIIa membrane protein expressed predominantly in prostate cells in normal human tissues. Structurally, STRAP-1 is a 339 amino acid protein characterized by a molecular topology of six transmembrane domains and intracellular N- and C-termini, suggesting that it folds in a "serpentine" manner into three extracellular and two intracellular loops. STRAP-1 protein expression is maintained at high levels across various stages of prostate cancer. Moreover, STRAP-1 is highly over-expressed in certain other human cancers.

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**NOVEL SERPENTINE TRANSMEMBRANE ANTIGENS  
EXPRESSED IN HUMAN CANCERS AND USES THEREOF**

**FIELD OF THE INVENTION**

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The invention described herein relates to a family of novel genes and their encoded proteins and tumor antigens, termed STRAPs, and to diagnostic and therapeutic methods and compositions useful in the management of various cancers, particularly including prostate cancer, colon cancer, bladder cancer, ovarian cancer and pancreatic cancer.

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**BACKGROUND OF THE INVENTION**

15      Cancer is the second leading cause of human death next to coronary disease. Around the world, millions of people die from cancer every year. In the United States alone, cancer cause the death of well over a half-million people each year, with some 1.4 million new cases diagnosed per year. While deaths from heart disease have been declining significantly, those resulting from cancer generally are on the rise. In the early part of the next century, cancer is predicted to become the leading cause of death.

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Worldwide, several cancers stand out as the leading killers. In particular, carcinomas of the lung, prostate, breast, colon, pancreas, and ovary represent the leading causes of cancer death. These and virtually all other carcinomas share a common lethal feature. With very few exceptions, metastatic disease from a carcinoma is fatal. Moreover, even for those cancer patients that initially survive their primary cancers, common experience has shown that their lives are dramatically altered. Many cancer patients experience strong anxieties driven by the awareness of the potential for recurrence or treatment failure. Many cancer patients experience physical debilitations following treatment.

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Generally speaking, the fundamental problem in the management of the deadliest cancers is the lack of effective and non-toxic systemic therapies. Molecular medicine, still very much in its infancy, promises to redefine the ways in which these cancers are managed. Unquestionably, there is an intensive worldwide effort aimed at the development of novel molecular approaches to cancer diagnosis and treatment. For example, there is a great interest in identifying truly tumor-specific genes and proteins that could be used as diagnostic and prognostic markers and/or therapeutic targets or agents. Research efforts in these areas are encouraging, and the increasing availability of useful molecular technologies has accelerated the acquisition of meaningful knowledge about cancer. Nevertheless, progress is slow and generally uneven.

Recently, there has been a particularly strong interest in identifying cell surface tumor-specific antigens which might be useful as targets for various immunotherapeutic or small molecule treatment strategies. A large number of such cell-surface antigens have been reported, and some have proven to be reliably associated with one or more cancers. Much attention has been focused on the development of novel therapeutic strategies which target these antigens. However, few truly effective immunological cancer treatments have resulted.

The use of monoclonal antibodies to tumor-specific or over-expressed antigens in the treatment of solid cancers is instructive. Although antibody therapy has been well researched for some 20 years, only very recently have corresponding pharmaceuticals materialized. One example is the humanized anti-HER2/neu monoclonal antibody, Herceptin, recently approved for use in the treatment of metastatic breast cancers overexpressing the HER2/neu receptor. Another is the human/mouse chimeric anti-CD20/B cell lymphoma antibody, Rituxan, approved for the treatment of non-Hodgkin's lymphoma. Several other antibodies are being evaluated for the treatment of cancer in clinical trials or in pre-clinical research, including a fully human IgG2 monoclonal antibody specific for the epidermal growth factor receptor (Yang et al., 1999, *Cancer Res.* 59: 1236). Evidently, antibody therapy is finally emerging from a long embryonic phase. Nevertheless, there is still a very great need for new, more-specific tumor antigens for the application of antibody and other biological therapies. In addition, there is a corresponding need for tumor antigens which may be useful as markers for antibody-based diagnostic and imaging methods, hopefully leading to the development of earlier diagnosis and greater prognostic precision.

As discussed below, the management of prostate cancer serves as a good example of the limited extent to which molecular biology has translated into real progress in the clinic. With limited exceptions, the situation is more or less the same for the other major carcinomas mentioned above.

Worldwide, prostate cancer is the fourth most prevalent cancer in men. In North America and Northern Europe, it is by far the most common male cancer and is the second leading cause of cancer death in men. In the United States alone, well over 40,000 men die annually of this disease, second only to lung cancer. Despite the magnitude of these figures, there is still no effective treatment for metastatic prostate cancer. Surgical prostatectomy, radiation therapy, hormone ablation therapy, and chemotherapy remain as the main treatment modalities. Unfortunately, these treatments are clearly ineffective for many. Moreover, these treatments are often associated with significant undesirable consequences.

On the diagnostic front, the serum PSA assay has been a very useful tool. Nevertheless, the specificity and general utility of PSA is widely regarded as lacking in several respects. Neither PSA testing, nor any other test nor biological marker has been proven capable of reliably identifying early-stage disease. Similarly, there is no marker available 5 for predicting the emergence of the typically fatal metastatic stage of the disease. Diagnosis of metastatic prostate cancer is achieved by open surgical or laparoscopic pelvic lymphadenectomy, whole body radionuclide scans, skeletal radiography, and/or bone lesion biopsy analysis. Clearly, better imaging and other less invasive diagnostic methods offer the promise of easing the difficulty those procedures place on a patient, as 10 well as improving therapeutic options. However, until there are prostate tumor markers capable of reliably identifying early-stage disease, predicting susceptibility to metastasis, and precisely imaging tumors, the management of prostate cancer will continue to be extremely difficult. Accordingly, more specific molecular tumor markers are clearly needed in the management of prostate cancer.

15 There are some known markers which are expressed predominantly in prostate, such as prostate specific membrane antigen (PSM), a hydrolase with 85% identity to a rat neuropeptidase (Carter et al., 1996, Proc. Natl. Acad. Sci. USA 93: 749; Bzdega et al., 1997, J. Neurochem. 69: 2270). However, the expression of PSM in small intestine and 20 brain (Israeli et al., 1994, Cancer Res. 54: 1807), as well its potential role in neuropeptide catabolism in brain, raises concern of potential neurotoxicity with anti-PSM therapies. Preliminary results using an Indium-111 labeled, anti-PSM monoclonal antibody to image 25 recurrent prostate cancer show some promise (Sodee et al., 1996, Clin Nuc Med 21: 759-766). More recently identified prostate cancer markers include PCTA-1 (Su et al., 1996, Proc. Natl. Acad. Sci. USA 93: 7252) and prostate stem cell antigen (PSCA) (Reiter et al., 1998, Proc. Natl. Acad. Sci. USA 95: 1735). PCTA-1, a novel galectin, is largely secreted 30 into the media of expressing cells and may hold promise as a diagnostic serum marker for prostate cancer (Su et al., 1996). PSCA, a GPI-linked cell surface molecule, was cloned from LAPC-4 cDNA and is unique in that it is expressed primarily in basal cells of normal prostate tissue and in cancer epithelia (Reiter et al., 1998). Vaccines for prostate cancer are also being actively explored with a variety of antigens, including PSM and PSA.

#### SUMMARY OF THE INVENTION

35 The present invention relates to a novel family of cell surface serpentine transmembrane antigens. Two of the proteins in this family are exclusively or predominantly expressed in the prostate, as well as in prostate cancer, and thus members of this family have been termed "STRAP" (Serpentine TRansmembrane Antigens of the Prostate). Four particular human STRAPs are described and characterized herein. The human STRAPs exhibit a

high degree of structural conservation among them but show no significant structural homology to any known human proteins.

The prototype member of the STRAP family, STRAP-1, appears to be a type IIIa membrane protein expressed predominantly in prostate cells in normal human tissues. Structurally, STRAP-1 is a 339 amino acid protein characterized by a molecular topology of six transmembrane domains and intracellular N- and C- termini, suggesting that it folds in a "serpentine" manner into three extracellular and two intracellular loops. STRAP-1 protein expression is maintained at high levels across various stages of prostate cancer. Moreover, STRAP-1 is highly over-expressed in certain other human cancers. In particular, cell surface expression of STRAP-1 has been definitively confirmed in a variety of prostate and prostate cancer cells, bladder cancer cells and colon cancer cells. These characteristics indicate that STRAP-1 is a specific cell-surface tumor antigen expressed at high levels in prostate, bladder, colon, and other cancers.

STRAP-2, STRAP-3 and STRAP-4 are also described herein. All are structurally related, but show unique expression profiles. STRAP-2, like STRAP-1, is prostate-specific in normal human tissues and is also expressed in prostate cancer. In contrast, STRAP-3 and STRAP-4 appear to show a different restricted expression pattern.

The invention provides polynucleotides corresponding or complementary to all or part of the STRAP genes, mRNAs, and/or coding sequences, preferably in isolated form, including polynucleotides encoding STRAP proteins and fragments thereof, DNA, RNA, DNA/RNA hybrid, and related molecules, polynucleotides or oligonucleotides complementary to the STRAP genes or mRNA sequences or parts thereof, and polynucleotides or oligonucleotides which hybridize to the STRAP genes, mRNAs, or to STRAP-encoding polynucleotides. Also provided are means for isolating cDNAs and the genes encoding STRAPs. Recombinant DNA molecules containing STRAP polynucleotides, cells transformed or transduced with such molecules, and host-vector systems for the expression of STRAP gene products are also provided. The invention further provides STRAP proteins and polypeptide fragments thereof. The invention further provides antibodies that bind to STRAP proteins and polypeptide fragments thereof, including polyclonal and monoclonal antibodies, murine and other mammalian antibodies, chimeric antibodies, humanized and fully human antibodies, and antibodies labeled with a detectable marker, and antibodies conjugated to radionuclides, toxins or other therapeutic compositions. The invention further provides methods for detecting the presence of STRAP polynucleotides and proteins in various biological samples, as well as methods for identifying cells that express a STRAP. The invention further provides various

therapeutic compositions and strategies for treating prostate cancer, including particularly, antibody, vaccine and small molecule therapy.

5 BRIEF DESCRIPTION OF THE FIGURES

10 FIG. 1. STRAP-1 structure. 1A: Nucleotide and deduced amino acid sequences of STRAP-1 (8P1B4) clone 10 cDNA (SEQ ID NOS. XX and XX, respectively). The start Methionine is indicated in bold at amino acid residue position 1 and six putative transmembrane domains are indicated in bold and are underlined. 1B: Schematic representation of STRAP-1 transmembrane orientation; amino acid residues bordering the predicted extracellular domains are indicated and correspond to the numbering scheme of FIG. 1A. 1C: G/C rich 5' non-coding sequence of the STRAP-1 gene as determined by overlapping sequences of clone 10 and clone 3.

15 FIG. 2. Predominant expression of STRAP-1 in prostate tissue. First strand cDNA was prepared from 16 normal tissues, the LAPC xenografts (4AD, 4AI and 9AD) and HeLa cells. Normalization was performed by PCR using primers to actin and GAPDH. Semi-quantitative PCR, using primers derived from STRAP-1 (8P1D4) cDNA (FIG. 1A), shows predominant expression of STRAP-1 in normal prostate and the LAPC xenografts. The following primers were used to amplify STRAP-1:

8P1D4.1 5' ACTTTGTTGATGACCAGGATTGGA 3' (SEQ ID NO: XX)  
8P1D4.2 5' CAGAACTTCAGCACACACAGGAAC 3' (SEQ ID NO: XX)

20 25 FIG. 3. Northern blot analyses of STRAP-1 expression in various normal human tissues and prostate cancer xenografts, showing predominant expression of STRAP-1 in prostate tissue. FIG. 3A: Two multiple tissue northern blots (Clontech) were probed with a full length STRAP cDNA clone 10 (FIG. 1A; SEQ ID NO: XX). Size standards in kilobases (kb) are indicated on the side. Each lane contains 2 µg of mRNA that was normalized by using a β-actin probe. FIG. 3B: Multiple tissue RNA dot blot (Clontech, Human Master Blot cat# 7770-1) probed with STRAP-1 cDNA clone 10 (FIG. 1A; SEQ ID NO: XX), showing approximately five-fold greater expression in prostate relative to other tissues with significant detectable expression.

30 35 FIG. 4. Nucleotide sequence of STRAP-1 GTH9 clone (SEQ ID NO: XX) corresponding to the 4 kb message on northern blots (FIG. 3A). The sequence contains an intron of 2399 base pairs relative to the STRAP-1 clone 10 sequence of FIG. 1A; coding regions are nucleotides 96-857 and 3257- 3510 (indicated in bold). The start ATG is in bold and

underlined, the STOP codon is in bold and underlined, and the intron-exon boundaries are underlined.

5 FIG. 5. Expression of STRAP-1 in prostate and multiple cancer cell lines and prostate cancer xenografts. Xenograft and cell line filters were prepared with 10 µg of total RNA per lane. The blots were analyzed using the STRAP-1 clone 10 as probe. All RNA samples were normalized by ethidium bromide staining and subsequent analysis with a  $\beta$ -actin probe. FIG. 5A: Expression in various cancer cell lines and xenografts and prostate. Lanes as follows: (1) PrEC cells, (2) normal prostate tissue, (3) LAPC-4 AD 10 xenograft, (4) LAPC-4 AI xenograft, (5) LAPC-9 AD xenograft, (6) LAPC-9 AI xenograft, (7) LNCaP cells, (8) PC-3 cells, (9) DU145 cells, (10) PANC-1 cells, (11) BxPC-3 cells, (12) HPAC cells, (13) Capan-1 cells, (14) CACO-2 cells, (15) LOVO cells, (16) T84 cells, (17) COLO-205 cells, (18) KCL-22 cells (acute lymphocytic leukemia, ALL), (19) HT1197 cells, (20) SCABER cells, (21) UM-UC-3 cells, (22) TCCSUP cells, (23) J82 cells, (24) 5637 cells, 15 (25) RD-ES cells (Ewing sarcoma, EWS), (26) CAMA-1 cells, (27) DU4475 cells, (28) MCF-7 cells, (29) MDA-MB-435s cells, (30) NTERA-2 cells, (31) NCCIT cells, (32) TERA-1 cells, (33) TERA-2 cells, (34) A431 cells, (35) HeLa cells, (36) OV-1063 cells, (37) PA-1 cells, (38) SW 626 cells, (39) CAOV-3 cells. FIG. 5B: The expression of STRAP-1 in subcutaneously 20 (sc) grown LAPC xenografts compared to the expression in LAPC-4 and LAPC-9 xenografts grown in the tibia (it) of mice.

25 FIG. 6. Western blot analysis of STRAP-1 protein expression in tissues and multiple cell lines. Western blots of cell lysates prepared from prostate cancer xenografts and cell lines were probed with a polyclonal anti-STRAP-1 antibody preparation (see Example XX for details). The samples contain 20 µg of protein and were normalized with anti-Grb-2 probing of the Western blots.

30 FIG. 7. Cell surface biotinylation of STRAP-1. FIG. 7A: Cell surface biotinylation of 293T cells transfected with vector alone or with vector containing cDNA encoding 6His-tagged STRAP-1. Cell lysates were immunoprecipitated with specific antibodies, transferred to a membrane and probed with horseradish peroxidase-conjugated streptavidin. Lanes 1-4 and 6 correspond to immunoprecipitates from lysates prepared from STRAP-1 expressing 293T cells. Lanes 5 and 7 are immunoprecipitates from vector transfected cells. The immunoprecipitations were performed using the following antibodies: (1) 35 sheep non-immune, (2) anti-Large T antigen, (3) anti- CD71 (transferrin receptor), (4) anti-His, (5) anti-His, (6) anti-STRAP-1, (7) anti-STRAP-1. FIG. 7B: Prostate cancer (LNCaP, PC-3, DU145), bladder cancer (UM-UC-3, TCCSUP) and colon cancer (LOVO, COLO) cell lines were either biotinylated (+) or not (-) prior to lysis. Western blots of streptavidin-gel

purified proteins were probed with anti-STRAP-1 antibodies. Molecular weight markers are indicated in kilodaltons (kD).

5 FIG. 8. Immunohistochemical analysis of STRAP-1 expression using anti-STRAP-1 polyclonal antibody. Tissues were fixed in 10% formalin and embedded in paraffin. Tissue sections were stained using anti-STRAP-1 polyclonal antibodies directed towards the N-terminal peptide. Samples include: (a) LNCaP cells probed in the presence of N-terminal STRAP-1 peptide 1, (b) LNCaP plus non specific peptide 2, (c) normal prostate tissue, (d) grade 3 prostate carcinoma, (e) grade 4 prostate carcinoma, (f) LAPC-9 AD 10 xenograft, (g) normal bladder, (h) normal colon. All images are at 400x magnification.

15 FIG. 9. Partial nucleotide and deduced amino acid sequences of STRAP-2 (98P4B6) clone GTA3 cDNA (SEQ ID NO: XX). The 5' end sequence of this clone contains an ORF of 173 amino acids.

20 FIG. 10. Nucleotide sequences of additional STRAP family members identified by searching the dbest database with the protein sequence of STRAP-1. In addition to STRAP-1, another three STRAP family members are indicated with their GenBank accession numbers. One of these corresponds to the gene 98P4B6 that was identified by SSH.

25 FIG. 11. Primary structural comparison of STRAP family proteins. FIG. 11A. Amino acid sequence alignment of STRAP-1 (8P1D4 CLONE 10; SEQ ID NO: XX) and STRAP-2 (98P4B6; SEQ ID NO: XX) sequences. The alignment was performed using the SIM alignment program of the Baylor College of Medicine Search Launcher Web site. Results show a 61.4% identity in a 171 amino acid overlap; Score: 576.0; Gap frequency: 0.0%. FIG. 11B. Amino acid sequence alignment of STRAP-1 with partial ORF sequences of STRAP-2 and two other putative family member proteins using PIMA program (PIMA 1.4 program at Internet address <<http://dot.imegen.bcm.tmc.edu:9331/multi-align/multi-align.html>>); transmembrane domains identified by the SOSUI program (available at Internet address <[http://www.tuat.ac.jp/~mitaku/adv\\_sosui/submit.html](http://www.tuat.ac.jp/~mitaku/adv_sosui/submit.html)>). 30 are in bold.

35 FIG. 12. Predominant expression of AI139607 in placenta and prostate. First strand cDNA was prepared from 16 normal tissues. Normalization was performed by PCR using primers to actin and GAPDH. Semi-quantitative PCR, using primers to AI139607, shows predominant expression of AI139607 in placenta and prostate after 25 cycles of amplification. The following primers were used to amplify AI139607:

AI139607.1 5' TTAGGACAACTTGATCACCAGCA 3'

(SEQ ID NO: XX)

AI139607.2 5' TGTCCAGTCCAAACTGGGTTATT 3' (SEQ ID NO: XX)

FIG. 13. Predominant expression of R80991 in liver. First strand cDNA was prepared from 16 normal tissues. Normalization was performed by PCR using primers to actin and GAPDH. Semi-quantitative PCR, using primers to R80991, shows predominant expression of R80991 in liver after 25 cycles of amplification. The following primers were used to amplify R80991:

R80991.1 5' AGGGAGTTCAGCTTCGTTAGTC 3' (SEQ ID NO: XX)

R80991.2 5' GGTAGAACTTGTAGCGGCTCTCCT 3' (SEQ ID NO: XX)

FIG. 14. Predominant expression of STRAP-2 (98P4B6) in prostate tissue. First strand cDNA was prepared from 8 normal tissues, the LAPC xenografts (4AD, 4AI and 9AD) and HeLa cells. Normalization was performed by PCR using primers to actin and GAPDH. Semi-quantitative PCR, using primers to 98P4B6, shows predominant expression of 98P4B6 in normal prostate and the LAPC xenografts. The following primers were used to amplify STRAP II:

98P4B6.1 5' GACTGAGCTGGAACCTGGAATTGT 3' (SEQ ID NO: XX)

98P4B6.2 5' TTTGAGGAGACTTCATCTCACTGG 3' (SEQ ID NO: XX)

FIG. 15. Lower expression of the prostate-specific STRAP-2/98P4B6 gene in prostate cancer xenografts determined by Northern blot analysis. Human normal tissue filters (A and B) were obtained from CLONTECH and contain 2 µg of mRNA per lane. Xenograft filter (C) was prepared with 10 µg of total RNA per lane. The blots were analyzed using the SSH derived 98P4B6 clone as probe. All RNA samples were normalized by ethidium bromide staining.

FIG. 16. Expression of STRAP-2 in prostate and select cancer cell lines as determined by Northern blot analysis. Xenograft and cell line filters were prepared with 10 µg total RNA per lane. The blots were analyzed using an SSH derived 98P4B6 clone as probe. All RNA samples were normalized by ethidium bromide staining.

FIG. 17. Chromosomal localization of STRAP family members. The chromosomal localizations of the STRAP genes described herein were determined using the GeneBridge4 radiation hybrid panel (Research Genetics, Huntsville AL). The mapping for STRAP-2 and AI139607 was performed using the Stanford G3 radiation hybrid panel (Research Genetics, Huntsville AL).

FIG. 18. Schematic representation of Intron-Exon boundaries within the ORF of human STRAP-1 gene. A total of 3 introns (i) and 4 exons (e) were identified.

FIG. 19. Zooblot southern analysis of STRAP-1 gene in various species. Genomic DNA was prepared from several different organisms including human, monkey, dog, mouse, chicken and *Drosophila*. Ten micrograms of each DNA sample was digested with EcoRI, blotted onto nitrocellulose and probed with a STRAP-1 probe. Size standards are indicated on the side in kilobases (kb).

FIG. 20. Southern blot analysis of mouse BAC with a STRAP-1 probe. DNA was prepared from human cells to isolate genomic DNA and from a mouse BAC clone (12P11) that contains the mouse STRAP gene. Each DNA sample was digested with EcoRI, blotted onto nitrocellulose and probed. Eight micrograms of genomic DNA was compared to 250 ng of mouse BAC DNA.

15 **DETAILED DESCRIPTION OF THE INVENTION**

Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 2nd. edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

30 As used herein, the terms "advanced prostate cancer", "locally advanced prostate cancer", "advanced disease" and "locally advanced disease" mean prostate cancers which have extended through the prostate capsule, and are meant to include stage C disease under the American Urological Association (AUA) system, stage C1 - C2 disease  
35 under the Whitmore-Jewett system, and stage T3 - T4 and N+ disease under the TNM (tumor, node, metastasis) system. In general, surgery is not recommended for patients with locally advanced disease, and these patients have substantially less favorable outcomes compared to patients having clinically localized (organ-confined) prostate cancer. Locally advanced disease is clinically identified by palpable evidence of

induration beyond the lateral border of the prostate, or asymmetry or induration above the prostate base. Locally advanced prostate cancer is presently diagnosed pathologically following radical prostatectomy if the tumor invades or penetrates the prostatic capsule, extends into the surgical margin, or invades the seminal vesicles.

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As used herein, the terms "metastatic prostate cancer" and "metastatic disease" mean prostate cancers which have spread to regional lymph nodes or to distant sites, and are meant to include stage D disease under the AUA system and stage TxNxM+ under the TNM system. As is the case with locally advanced prostate cancer, surgery is generally 10 not indicated for patients with metastatic disease, and hormonal (androgen ablation) therapy is the preferred treatment modality. Patients with metastatic prostate cancer eventually develop an androgen-refractory state within 12 to 18 months of treatment initiation, and approximately half of these patients die within 6 months thereafter. The most common site for prostate cancer metastasis is bone. Prostate cancer bone 15 metastases are, on balance, characteristically osteoblastic rather than osteolytic (i.e., resulting in net bone formation). Bone metastases are found most frequently in the spine, followed by the femur, pelvis, rib cage, skull and humerus. Other common sites for metastasis include lymph nodes, lung, liver and brain. Metastatic prostate cancer is typically diagnosed by open or laparoscopic pelvic lymphadenectomy, whole body 20 radionuclide scans, skeletal radiography, and/or bone lesion biopsy.

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As used herein, the term "polynucleotide" means a polymeric form of nucleotides of at least 10 bases or base pairs in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide, and is meant to include single and double stranded forms of DNA.

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As used herein, the term "polypeptide" means a polymer of at least 10 amino acids. Throughout the specification, standard three letter or single letter designations for amino acids are used.

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As used herein, the terms "hybridize", "hybridizing", "hybridizes" and the like, used in the context of polynucleotides, are meant to refer to conventional hybridization conditions, preferably such as hybridization in 50% formamide/6XSSC/0.1% SDS/100 µg/ml ssDNA, in which temperatures for hybridization are above 37 degrees C and temperatures for washing in 0.1XSSC/0.1% SDS are above 55 degrees C, and most preferably to stringent hybridization conditions.

In the context of amino acid sequence comparisons, the term "identity" is used to express the percentage of amino acid residues at the same relative position which are the same.

Also in this context, the term "homology" is used to express the percentage of amino acid residues at the same relative positions which are either identical or are similar, using the conserved amino acid criteria of BLAST analysis, as is generally understood in the art. Further details regarding amino acid substitutions, which are considered conservative under such criteria, are provided below.

5 Additional definitions are provided throughout the subsections which follow.

#### MOLECULAR AND BIOCHEMICAL FEATURES OF THE STRAPs

10 The invention relates to a novel family of proteins, termed STRAPs. Four STRAPs are specifically described herein by way of structural, molecular and biochemical features. As is further described in the Examples which follow, the STRAPs have been characterized in a variety of ways. For example, analyses of nucleotide coding and amino acid sequences were conducted in order to identify conserved structural 15 elements within the STRAP family. Extensive RT-PCR and Northern blot analyses of STRAP mRNA expression were conducted in order to establish the range of normal and cancerous tissues expressing the various STRAP messages. Western blot, immunohistochemical and flow cytometric analyses of STRAP protein expression were conducted to determine protein expression profiles, cell surface localization and gross 20 molecular topology of STRAP.

The prototype member of the STRAP family, STRAP-1, is a six-transmembrane cell surface protein of 339 amino acids with no identifiable homology to any known human protein. The cDNA nucleotide and deduced amino acid sequences of human STRAP-1 25 are shown in FIG. 1A. A gross topological schematic of the STRAP-1 protein integrated within the cell membrane is shown in FIG. 1B. STRAP-1 expression is predominantly prostate-specific in normal tissues. Specifically, extensive analysis of STRAP-1 mRNA and protein expression in normal human tissues shows that STRAP-1 protein is predominantly expressed in prostate and, to a far smaller degree, in bladder. STRAP-1 30 mRNA is also relatively prostate specific, with only very low level expression detected in a few other normal tissues. In cancer, STRAP-1 mRNA and protein is consistently expressed at high levels in prostate cancer and during all stages of the disease. STRAP-1 is also expressed in other cancers. Specifically, STRAP-1 mRNA is expressed at very high levels in bladder, colon, pancreatic, and ovarian cancer (as well as other cancers). 35 In addition, cell surface expression of STRAP-1 protein has been established in prostate, bladder and colon cancers. Therefore, STRAP-1 has all of the hallmark characteristics of an excellent therapeutic target for the treatment of certain cancers, including particularly prostate, colon and bladder carcinomas.

STRAP-2 is a highly homologous transmembrane protein encoded by a distinct gene. The STRAP-1 and STRAP-2 sequences show a high degree of structural conservation, particularly throughout their predicted transmembrane domains. The partial cDNA nucleotide and deduced amino acid sequences of STRAP-2 are shown in FIG. 9. Both the 5 STRAP-1 and STRAP-2 genes are located on chromosome 7, but on different arms. STRAP-2 exhibits a markedly different mRNA and protein expression profile relative to STRAP-1, suggesting that these two STRAP family members may be differentially regulated. STRAP-2 appears to be very prostate-specific, as significant mRNA expression is not detected in a variety of normal tissues. In prostate cancer, STRAP-2 10 also appears to follow a different course relative to STRAP-1, since STRAP-2 expression is down-regulated in at least some prostate cancers. In addition, STRAP-2 expression in other non-prostate cancers tested seems generally absent, although high level expression of STRAP-2 (like STRAP-1) is detected in Ewing sarcoma.

15 STRAP-3 and STRAP-4 appear to be closely related to both STRAP-1 and STRAP-2 on a structural level, and both appear to be transmembrane proteins as well. STRAP-3 and STRAP-4 show unique expression profiles. STRAP-3, for example, appears to have an expression pattern which is predominantly restricted to placenta and, to a smaller degree, expression is seen in prostate but not in other normal tissues tested. STRAP-4 20 seems to be expressed predominantly in liver. Neither STRAP-3 nor STRAP-4 appear to be expressed in prostate cancer xenografts which exhibit high level STRAP-1 and STRAP-2 expression.

25 Three of the four STRAPs described herein map to human chromosome 7 (STRAP-1, -2 and 3). Interestingly, STRAP-1 maps within 7p22 (7p22.3), a large region of allelic gain reported for both primary and recurrent prostate cancers (Visakorpi et al., 1995 *Cancer Res.* 55: 342, Nupponen et al., 1998 *American J. Pathol.* 153: 141), suggesting that up-regulation of STRAP-1 in cancer might include genomic mechanisms.

30 The function of the STRAPs are not known. Other cell surface molecules that contain six transmembrane domains include ion channels (Dolly and Parcej, 1996 *J Bioenerg Biomembr* 28:231) and water channels or aquaporins (Reizer et al., 1993 *Crit Rev Biochem Mol Biol* 28:235). Structural studies show that both types of molecules assemble into tetrameric complexes to form functional channels (Christie, 1995, *Clin Exp Pharmacol Physiol* 22:944, Walz et al., 1997 *Nature* 387:624, Cheng et al., 1997 *Nature* 387:627). Immunohistochemical staining of STRAP-1 in the prostate gland seems to be 35 concentrated at the cell-cell boundaries, with less staining detected at the luminal side. This may suggest a role for STRAP-1 in tight-junctions, gap-junctions or cell adhesion. In order to test these possibilities, *xenopus* oocytes (or other cells) expressing STRAP may

being analyzed using voltage-clamp and patch-clamp experiments to determine if STRAP functions as an ion-channel. Oocyte cell volume may also be measured to determine if STRAP exhibits water channel properties. If STRAPs function as channel or gap-junction proteins, they may serve as excellent targets for inhibition using, for example, antibodies, 5 small molecules, and polynucleotides capable of inhibiting expression or function. The restricted expression pattern in normal tissue, and the high levels of expression in cancer tissue suggest that interfering with STRAP function may selectively kill cancer cells.

Since the STRAP gene family is predominantly expressed in epithelial tissue, it seems 10 possible that the STRAP proteins function as ion channels or gap-junction proteins in epithelial cell function. Ion channels have been implicated in proliferation and invasiveness of prostate cancer cells (Lalani et al., 1997, *Cancer Metastasis Rev* 16:29). Both rat and human prostate cancer cells contain sub-population of cells with higher and lower expression levels of sodium channels. Higher levels of sodium channel expression 15 correlate with more aggressive invasiveness in vitro (Smith et al., 1998, *FEBS Lett.* 423:19). Similarly, it has been shown that a specific blockade of sodium channels inhibits the invasiveness of PC-3 cells in vitro (Laniado et al., 1997, *Am. J. Pathol.* 150:1213), while specific inhibition of potassium channels in LNCaP cells inhibited cell proliferation 20 (Skryma et al., 1997, *Prostate* 33:112). These reports suggest a role for ion channels in prostate cancer and also demonstrate that small molecules that inhibit ion channel function may interfere with prostate cancer proliferation.

#### STRAP POLYNUCLEOTIDES

One aspect of the invention provides polynucleotides corresponding or complementary 25 to all or part of a STRAP gene, mRNA, and/or coding sequence, preferably in isolated form, including polynucleotides encoding a STRAP protein and fragments thereof, DNA, RNA, DNA/RNA hybrid, and related molecules, polynucleotides or oligonucleotides complementary to a STRAP gene or mRNA sequence or a part thereof, and polynucleotides or oligonucleotides which hybridize to a STRAP gene, mRNA, or to a 30 STRAP-encoding polynucleotide (collectively, "STRAP polynucleotides"). As used herein, STRAP genes and proteins are meant to include the STRAP-1 and STRAP-2 genes and proteins, the genes and proteins corresponding to GeneBank Accession numbers AI139607 and R80991 (STRAP-3 and STRAP-4, respectively), and the genes and proteins corresponding to other STRAP proteins and structurally similar variants of the foregoing. 35 Such other STRAP proteins and variants will generally have coding sequences which are highly homologous to the STRAP-1 and/or STRAP-2 coding sequences, and preferably will share at least about 50% amino acid identity and at least about 60% amino acid homology (using BLAST criteria), more preferably sharing 70% or greater homology (using BLAST criteria).

The STRAP family member gene sequences described herein encode STRAP proteins - sharing unique highly conserved amino acid sequence domains which distinguish them from other proteins. Proteins which include one or more of these unique highly 5 conserved domains may be related to the STRAP family members or may represent new STRAP proteins. Referring to FIG. 11A, which is an amino acid sequence alignment of the full STRAP-1 and partial STRAP-2 protein sequences, the STRAP-1 and STRAP-2 sequences share 61% identity and 79% homology, with particularly close sequence conservation in the predicted transmembrane domains. Referring to FIG. 11B, which is 10 an amino acid alignment of the available structures of the four STRAP family members, very close conservation is apparent in the overlapping regions, particularly in the fourth and fifth transmembrane domains and the predicted intracellular loop between them. Amino acid sequence comparisons show that (1) STRAP-2 and STRAP-3 are 50% identical and 69% homologous in their overlapping sequences; (2) STRAP-2 and STRAP-4 15 are 56% identical and 87% homologous in their overlapping sequences; (3) STRAP-3 and STRAP-1 are 37% identical and 63% homologous in their overlapping sequences; (4) STRAP-3 and STRAP-4 are 38% identical and 57% homologous in their overlapping sequences; and (5) STRAP 4 and STRAP-1 are 42% identical and 65% homologous in their overlapping sequences.

20 A STRAP polynucleotide may comprise a polynucleotide having the nucleotide sequence of human STRAP-1 as shown in FIG. 1A (SEQ ID NO. XX) or the nucleotide sequence of human STRAP-2 as shown in FIG. 9 (SEQ ID NO: XX), a sequence complementary to either 25 of the foregoing, or a polynucleotide fragment of any of the foregoing. Another embodiment comprises a polynucleotide which encodes the human STRAP-1 protein amino acid sequence as shown in FIG. 1A (SEQ ID NO. XX) or which encodes the human STRAP-2 protein amino acid sequence as shown in FIG. 9 (SEQ ID NO: XX), a sequence complementary to either of the foregoing, or a polynucleotide fragment of any of the foregoing. Another embodiment comprises a polynucleotide which is capable of 30 hybridizing under stringent hybridization conditions to the human STRAP-1 cDNA shown in FIG. 1A (SEQ ID NO. XX) or to a polynucleotide fragment thereof. Another embodiment comprises a polynucleotide which is capable of hybridizing under stringent hybridization conditions to the human STRAP-2 cDNA shown in FIG. 9 (SEQ ID NO. XX) or to a polynucleotide fragment thereof.

35 Specifically contemplated are genomic DNA, cDNAs, ribozymes, and antisense molecules, as well as nucleic acid molecules based on an alternative backbone or including alternative bases, whether derived from natural sources or synthesized. For example, antisense molecules can be RNAs or other molecules, including peptide nucleic acids (PNAs) or

non-nucleic acid molecules such as phosphorothioate derivatives, that specifically bind DNA or RNA in a base pair-dependent manner. A skilled artisan can readily obtain these classes of nucleic acid molecules using the STRAP polynucleotides and polynucleotide sequences disclosed herein.

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Further specific embodiments of this aspect of the invention include primers and primer pairs, which allow the specific amplification of the polynucleotides of the invention or of any specific parts thereof, and probes that selectively or specifically hybridize to nucleic acid molecules of the invention or to any part thereof. Probes may be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme. Such probes and primers can be used to detect the presence of a STRAP polynucleotide in a sample and as a means for detecting a cell expressing a STRAP protein. Examples of such probes include polypeptides comprising all or part of the human STRAP-1 cDNA sequence shown in FIG. 1A (SEQ ID NO. XX) and polypeptides comprising all or part of the human STRAP-2 cDNA sequence shown in FIG. 1A (SEQ ID NO. XX). Examples of primer pairs capable of specifically amplifying STRAP mRNAs are also described in the Examples which follow. As will be understood by the skilled artisan, a great many different primers and probes may be prepared based on the sequences provided in herein and used effectively to amplify and/or detect a STRAP mRNA or an mRNA encoding a particular STRAP family member (e.g., STRAP-1).

25

As used herein, a polynucleotide is said to be "isolated" when it is substantially separated from contaminant polynucleotides which correspond or are complementary to genes other than the STRAP gene or which encode polypeptides other than STRAP gene product or fragments thereof. A skilled artisan can readily employ nucleic acid isolation procedures to obtain an isolated STRAP polynucleotide.

30 35

The STRAP polynucleotides of the invention are useful for a variety of purposes, including but not limited to their use as probes and primers for the amplification and/or detection of the STRAP gene(s), mRNA(s), or fragments thereof; as reagents for the diagnosis and/or prognosis of prostate cancer and other cancers; as coding sequences capable of directing the expression of STRAP polypeptides; as tools for modulating or inhibiting the expression of the STRAP gene(s) and/or translation of the STRAP transcript(s); and as therapeutic agents.

#### METHODS FOR ISOLATING STRAP-ENCODING NUCLEIC ACID MOLECULES

The STRAP cDNA sequences described herein enable the isolation of other polynucleotides encoding STRAP gene product(s), as well as the isolation of polynucleotides encoding

STRAP gene product homologues, alternatively spliced isoforms, allelic variants, and mutant forms of the STRAP gene product. Various molecular cloning methods that can be employed to isolate full length cDNAs encoding a STRAP gene are well known (See, for example, Sambrook, J. et al., *Molecular Cloning: A Laboratory Manual*, 2d edition., Cold Spring Harbor Press, New York, 1989; *Current Protocols in Molecular Biology*. Ausubel et al., Eds., Wiley and Sons, 1995). For example, lambda phage cloning methodologies may be conveniently employed, using commercially available cloning systems (e.g., Lambda ZAP Express, Stratagene). Phage clones containing STRAP gene cDNAs may be identified by probing with a labeled STRAP cDNA or a fragment thereof. For example, in one embodiment, the STRAP-1 cDNA (FIG. 1A) or a portion thereof can be synthesized and used as a probe to retrieve overlapping and full length cDNAs corresponding to a STRAP gene. Similarly, the STRAP-2 cDNA sequence may be employed. A STRAP gene may be isolated by screening genomic DNA libraries, bacterial artificial chromosome libraries (BACs), yeast artificial chromosome libraries (YACs), and the like, with STRAP DNA probes or primers.

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#### RECOMBINANT DNA MOLECULES AND HOST-VECTOR SYSTEMS

The invention also provides recombinant DNA or RNA molecules containing a STRAP polynucleotide, including but not limited to phages, plasmids, phagemids, cosmids, YACs, BACs, as well as various viral and non-viral vectors well known in the art, and cells 20 transformed or transfected with such recombinant DNA or RNA molecules. As used herein, a recombinant DNA or RNA molecule is a DNA or RNA molecule that has been subjected to molecular manipulation in vitro. Methods for generating such molecules are well known (see, for example, Sambrook et al, 1989, *supra*).

25

The invention further provides a host-vector system comprising a recombinant DNA molecule containing a STRAP polynucleotide within a suitable prokaryotic or eukaryotic host cell. Examples of suitable eukaryotic host cells include a yeast cell, a plant cell, or an animal cell, such as a mammalian cell or an insect cell (e.g., a baculovirus-infectible cell such as an Sf9 cell). Examples of suitable mammalian cells include various prostate 30 cancer cell lines such LnCaP, PC-3, DU145, LAPC-4, TsuPr1, other transfectable or transducible prostate cancer cell lines, as well as a number of mammalian cells routinely used for the expression of recombinant proteins (e.g., COS, CHO, 293, 293T cells). More particularly, a polynucleotide comprising the coding sequence of a STRAP may be used to generate STRAP proteins or fragments thereof using any number of host-vector systems 35 routinely used and widely known in the art.

A wide range of host-vector systems suitable for the expression of STRAP proteins or fragments thereof are available, see for example, Sambrook et al., 1989, *supra*; *Current Protocols in Molecular Biology*, 1995, *supra*). Preferred vectors for mammalian expression

includ but ar not limited to pcDNA 3.1 myc-His-tag (Invitrogen) and the retroviral vector pSRatkneo (Muller et al., 1991, MCB 11:1785). Using these expr ssion vectors, STRAP may be preferably expressed in several prostate cancer and non-prostate cell lines, including for example 293, 293T, rat-1, 3T3, PC-3, LNCaP and TsuPr1. The host-vector 5 systems of the invention are useful for the production of a STRAP protein or fragment thereof. Such host-vector systems may be employed to study the functional properties of STRAP and STRAP mutations.

10 Proteins encoded by the STRAP genes, or by fragments thereof, will have a variety of uses, including but not limited to generating antibodies and in methods for identifying ligands and other agents and cellular constituents that bind to a STRAP gene product. Antibodies raised against a STRAP protein or fragment thereof may be useful in diagnostic and prognostic assays, imaging methodologies (including, particularly, cancer imaging), and therapeutic methods in the management of human cancers 15 characterized by expression of a STRAP protein, such as prostate, colon, breast, cervical and bladder carcinomas, ovarian cancers, testicular cancers and pancreatic cancers. Various immunological assays useful for the detection of STRAP proteins are contemplated, including but not limited to various types of radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), 20 immunocytochemical methods, and the like. Such antibodies may be labeled and used as immunological imaging reagents capable of detecting prostate cells (e.g., in radiosintigraphic imaging methods). STRAP proteins may also be particularly useful in generating cancer vaccines, ss further described below.

25 **STRAP PROTEINS**

Another aspect of the present invention provides various STRAP proteins and polypeptide fragments thereof. As used herein, a STRAP protein refers to a protein that has or includes the amino acid sequence of human STRAP-1 as provided in FIG. 1A (SEQ ID NO. XX), human STRAP-2 as provided in FIG. 9 (SEQ ID NO. XX), the amino acid sequence of other 30 mammalian STRAP homologues and variants, as well as allelic variants and conservative substitution mutants of these proteins that have STRAP biological activity.

The STRAP proteins of the invention include those specifically identified herein, as well as allelic variants, conservative substitution variants and homologs that can be 35 isolated/generated and characterized without undue experimentation following the methods outlined below. Fusion proteins which combine parts of different STRAP proteins or fragments thereof, as well as fusion proteins of a STRAP protein and a heterologous polypeptide are also included. Such STRAP proteins will be collectively referred to as the STRAP pr teins, the proteins of the invention, or STRAP. As used herein, the term "STRAP

"polypeptide" refers to a polypeptide fragment or a STRAP protein of at least 10 amino acids, preferably at least 15 amino acids.

A specific embodiment of a STRAP protein comprises a polypeptide having the amino acid sequence of human STRAP-1 as shown in FIG. 1A (SEQ ID NO. XX). Another embodiment of a STRAP protein comprises a polypeptide containing the partial STRAP-2 amino acid sequence as shown in FIG. 9 (SEQ ID NO. XX). Another embodiment comprises a polypeptide containing the partial STRAP-3 amino acid sequence of shown in FIG. 11B. Yet another embodiment comprises a polypeptide containing the partial STRAP-4 amino acid sequence of shown in FIG. 11B.

In general, naturally occurring allelic variants of human STRAP will share a high degree of structural identity and homology (e.g., 90% or more identity). Typically, allelic variants of the STRAP proteins will contain conservative amino acid substitutions within the STRAP sequences described herein or will contain a substitution of an amino acid from a corresponding position in a STRAP homologue. One class of STRAP allelic variants will be proteins that share a high degree of homology with at least a small region of a particular STRAP amino acid sequence, but will further contain a radical departure form the sequence, such as a non-conservative substitution, truncation, insertion or frame shift. Such alleles represent mutant STRAP proteins that typically do not perform the same biological functions or do not have all of the biological characteristics.

Conservative amino acid substitutions can frequently be made in a protein without altering either the conformation or the function of the protein. Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa. Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine (A) and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

STRAP proteins may be embodied in many forms, preferably in isolated form. As used herein, a protein is said to be "isolated" when physical, mechanical or chemical methods

are employed to remove the STRAP protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated STRAP protein. A purified STRAP protein molecule will be substantially free of other proteins or molecules which impair the binding of STRAP to antibody or other ligand. The nature and degree of isolation and purification will depend on the intended use. Embodiments of a STRAP protein include a purified STRAP protein and a functional, soluble STRAP protein. In one form, such functional, soluble STRAP proteins or fragments thereof retain the ability to bind antibody or other ligand.

5

10 The invention also provides STRAP polypeptides comprising biologically active fragments of the STRAP amino acid sequence, such as a polypeptide corresponding to part of the amino acid sequences for STRAP-1 as shown in FIG. 1A (SEQ ID NO. XX), STRAP-2 as shown in FIG. 9 (SEQ ID NO: XX), or STRAP-3 or STRAP-4 as shown in FIG. 11B. Such polypeptides of the invention exhibit properties of a STRAP protein, such as 15 the ability to elicit the generation of antibodies which specifically bind an epitope associated with a STRAP protein. Polypeptides comprising amino acid sequences which are unique to a particular STRAP protein (relative to other STRAP proteins) may be used to generate antibodies which will specifically react with that particular STRAP protein. For example, referring to the amino acid alignment of the STRAP-1 and STRAP-2 20 structures shown in FIG. 11A, the skilled artisan will readily appreciate that each molecule contains stretches of sequence unique to its structure. These unique stretches can be used to generate STRAP-1 or STRAP-2 specific antibodies.

25 STRAP polypeptides can be generated using standard peptide synthesis technology or using chemical cleavage methods well known in the art based on the amino acid sequences of the human STRAP proteins disclosed herein. Alternatively, recombinant methods can be used to generate nucleic acid molecules that encode a polypeptide fragment of a STRAP protein. In this regard, the STRAP-encoding nucleic acid molecules described herein provide means for generating defined fragments of STRAP proteins. STRAP polypeptides 30 are particularly useful in generating and characterizing domain specific antibodies (e.g., antibodies recognizing an extracellular or intracellular epitope of a STRAP protein), in generating STRAP family member specific antibodies (e.g., anti-STRAP-1, anti-STRAP 2 antibodies), identifying agents or cellular factors that bind to a particular STRAP or STRAP domain, and in various therapeutic contexts, including but not limited to cancer vaccines.

35 STRAP polypeptides containing particularly interesting structures can be predicted and/or identified using various analytical techniques well known in the art, including, for example, the methods of Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis, or on the basis of immunogenicity. Fragments containing such

structures are particularly useful in generating subunit specific anti-STRAP antibodies or in identifying cellular factors that bind to STRAP.

#### STRAP ANTIBODIES

5 Another aspect of the invention provides antibodies that bind to STRAP proteins and polypeptides. The most preferred antibodies will selectively bind to a STRAP protein and will not bind (or will bind weakly) to non-STRAP proteins and polypeptides. Anti-STRAP antibodies that are particularly contemplated include monoclonal and polyclonal antibodies as well as fragments containing the antigen binding domain and/or one or more

10 complementarity determining regions of these antibodies. As used herein, an antibody fragment is defined as at least a portion of the variable region of the immunoglobulin molecule which binds to its target, i.e., the antigen binding region.

For some applications, it may be desirable to generate antibodies which specifically react

15 with a particular STRAP protein and/or an epitope within a particular structural domain. For example, preferred antibodies useful for cancer therapy and diagnostic imaging purposes are those which react with an epitope in an extracellular region of the STRAP protein as expressed in cancer cells. Such antibodies may be generated by using the STRAP proteins described herein, or using peptides derived from predicted extracellular domains thereof,

20 as an immunogen. In this regard, with reference to the STRAP-1 protein topological schematic shown in FIG 1B, regions in the extracellular loops between the indicated transmembrane domains may be selected as used to design appropriate immunogens for raising extracellular-specific antibodies.

25 STRAP antibodies of the invention may be particularly useful in prostate cancer therapeutic strategies, diagnostic and prognostic assays, and imaging methodologies. The invention provides various immunological assays useful for the detection and quantification of STRAP and mutant STRAP proteins and polypeptides. Such assays generally comprise one or more STRAP antibodies capable of recognizing and binding a

30 STRAP or mutant STRAP protein, as appropriate, and may be performed within various immunological assay formats well known in the art, including but not limited to various types of radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), and the like. In addition, immunological imaging methods capable of detecting prostate cancer are also provided by the invention, including

35 but limited to radiosintigraphic imaging methods using labeled STRAP antibodies. Such assays may be clinically useful in the detection, monitoring, and prognosis of prostate cancer, particularly advanced prostate cancer.

STRAP antibodies may also be used in methods for purifying STRAP and mutant STRAP proteins and polypeptides and for isolating STRAP homologs and related molecules. For example, in one embodiment, the method of purifying a STRAP protein comprises incubating a STRAP antibody, which has been coupled to a solid matrix, with a lysate or other solution containing STRAP under conditions which permit the STRAP antibody to bind to STRAP; washing the solid matrix to eliminate impurities; and eluting the STRAP from the coupled antibody. Other uses of the STRAP antibodies of the invention include generating anti-idiotypic antibodies that mimic the STRAP protein.

STRAP antibodies may also be used therapeutically by, for example, modulating or inhibiting the biological activity of a STRAP protein or targeting and destroying prostate cancer cells expressing a STRAP protein. Antibody therapy of prostate and other cancers is more specifically described in a separate subsection below.

Various methods for the preparation of antibodies are well known in the art. For example, antibodies may be prepared by immunizing a suitable mammalian host using a STRAP protein, peptide, or fragment, in isolated or immunoconjugated form (Antibodies: A Laboratory Manual, CSH Press, Eds., Harlow, and Lane (1988); Harlow, Antibodies, Cold Spring Harbor Press, NY (1989)). In addition, fusion proteins of STRAP may also be used, such as a STRAP GST-fusion protein. In a particular embodiment, a GST fusion protein comprising all or most of the open reading frame amino acid sequence of FIG. 1A may be produced and used as an immunogen to generate appropriate antibodies. Cells expressing or overexpressing STRAP may also be used for immunizations. Similarly, any cell engineered to express STRAP may be used. Such strategies may result in the production of monoclonal antibodies with enhanced capacities for recognizing endogenous STRAP. Another useful immunogen comprises STRAP proteins linked to the plasma membrane of sheep red blood cells.

The amino acid sequence of STRAP as shown in FIG. 1A (SEQ ID NO. XX) may be used to select specific regions of the STRAP protein for generating antibodies. For example, hydrophobicity and hydrophilicity analyses of the STRAP amino acid sequence may be used to identify hydrophilic regions in the STRAP structure. Regions of the STRAP protein that show immunogenic structure, as well as other regions and domains, can readily be identified using various other methods known in the art, such as Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis. For the generation of antibodies which specifically recognize a mutant STRAP protein, amino acid sequences unique to the mutant (relative to wild type STRAP) are preferable.

Methods for preparing a protein or polypeptide for use as an immunogen and for preparing immunogenic conjugates of a protein with a carrier such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be used; in other instances linking reagents such as 5 those supplied by Pierce Chemical Co., Rockford, IL, may be effective. Administration of a STRAP immunogen is conducted generally by injection over a suitable time period and with use of a suitable adjuvant, as is generally understood in the art. During the immunization schedule, titers of antibodies can be taken to determine adequacy of antibody formation.

10 STRAP monoclonal antibodies are preferred and may be produced by various means well known in the art. For example, immortalized cell lines which secrete a desired monoclonal antibody may be prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by 15 immunoassay in which the antigen is the STRAP protein or STRAP fragment. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells may be expanded and antibodies produced either from in vitro cultures or from ascites fluid.

20 As mentioned above, numerous STRAP polypeptides may be used as immunogens for generating monoclonal antibodies using traditional methods. A particular embodiment comprises an antibody which immunohistochemically stains 293T cells transfected with an expression plasmid carrying the STRAP-1 coding sequence, the transfected cells expressing STRAP-1 protein, but does immunohistochemically stain untransfected 293T 25 cells. An assay for characterizing such antibodies is provided in Example 5 herein.

In another embodiment, STRAP-1 monoclonal antibodies may be generated using NIH 3T3 cells expressing STRAP-1 as an immunogen to generate mAbs that recognize the cell surface epitopes of STRAP-1. Reactive mAbs may be screened by cell-based ELISAs 30 using PC-3 cells over-expressing STRAP-1. In another specific embodiment, 3 peptides representing the extracellular regions of the STRAP-1 protein (specifically, REVIHPLATSHQQYFYKIPILV, RRSYRYKLLNWAYQQVQQNKEDAWIEHDVWRMEI and WIDIKQFVWYTPPTF) are coupled to sheep red blood cells for immunization. In another specific embodiment, recombinant STRAP-1 protein generated with an amino-terminal 35 His-tag using a suitable expression system (e.g., baculovirus expression system pBlueBac4.5, Invitrogen) is purified using a Nickel column and used as immunogen.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of the STRAP

protein can also be produced in the context of chimeric or CDR grafted antibodies of multiple species origin. Humanized or human STRAP antibodies may also be produced and are preferred for use in therapeutic contexts. Various approaches for producing such humanized antibodies are known, and include chimeric and CDR grafting methods; 5 methods for producing fully human monoclonal antibodies include phage display and transgenic methods (for review, see Vaughan et al., 1998, *Nature Biotechnology* 16: 535-539).

10 Fully human STRAP monoclonal antibodies may be generated using cloning technologies employing large human Ig gene combinatorial libraries (i.e., phage display) (Griffiths and Hoogenboom, *Building an in vitro immune system: human antibodies from phage display libraries*. In: *Protein Engineering of Antibody Molecules for Prophylactic and Therapeutic Applications in Man*. Clark, M. (Ed.), Nottingham Academic, pp 45-64 (1993); Burton and Barbas, *Human Antibodies from combinatorial libraries*. *Id.*, pp 65-82). Fully human STRAP 15 monoclonal antibodies may also be produced using transgenic mice engineered to contain human immunoglobulin gene loci as described in PCT Patent Application WO98/24893, Kucherlapati and Jakobovits et al., published December 3, 1997 (see also, Jakobovits, 1998, *Exp. Opin. Invest. Drugs* 7(4): 607-614). This method avoids the in vitro manipulation required with phage display technology and efficiently produces high affinity authentic 20 human antibodies.

25 Reactivity of STRAP antibodies with a STRAP protein may be established by a number of well known means, including Western blot, immunoprecipitation, ELISA, and FACS analyses using, as appropriate, STRAP proteins, peptides, STRAP-expressing cells or extracts thereof.

30 A STRAP antibody or fragment thereof of the invention may be labeled with a detectable marker or conjugated to a second molecule, such as a cytotoxic agent, and used for targeting the second molecule to a STRAP positive cell (Vitetta, E.S. et al., 1993, *Immunotoxin therapy*, in DeVita, Jr., V.T. et al., eds, *Cancer: Principles and Practice of Oncology*, 4th ed., J.B. Lippincott Co., Philadelphia, 2624-2636). Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme.

35 **METHODS FOR THE DETECTION OF STRAP**

Another aspect of the present invention relates to methods for detecting STRAP polynucleotides and STRAP proteins, as well as methods for identifying a cell which expresses STRAP.

More particularly, the invention provides assays for the detection of STRAP polynucleotides in a biological sample, such as serum, bone, prostate, and other tissues, urine, semen, cell preparations, and the like. Detectable STRAP polynucleotides include, for example, a STRAP gene or fragments thereof, STRAP mRNA, alternative splice variant STRAP mRNAs, 5 and recombinant DNA or RNA molecules containing a STRAP polynucleotide. A number of methods for amplifying and/or detecting the presence of STRAP polynucleotides are well known in the art and may be employed in the practice of this aspect of the invention.

In one embodiment, a method for detecting a STRAP mRNA in a biological sample 10 comprises producing cDNA from the sample by reverse transcription using at least one primer; amplifying the cDNA so produced using a STRAP polynucleotides as sense and antisense primers to amplify STRAP cDNAs therein; and detecting the presence of the amplified STRAP cDNA. In another embodiment, a method of detecting a STRAP gene in a biological sample comprises first isolating genomic DNA from the sample; amplifying 15 the isolated genomic DNA using STRAP polynucleotides as sense and antisense primers to amplify the STRAP gene therein; and detecting the presence of the amplified STRAP gene. Any number of appropriate sense and antisense probe combinations may be designed from the nucleotide sequences provided for STRAP-1 (FIG. 1A; SEQ ID NO. XX), STRAP-2 (FIG. 9; SEQ ID NO. XX), STRAP-3 (FIG. 10; SEQ ID NO. XX), or STRAP-4 (FIG. 10; 20 SEQ ID NO. XX), as appropriate, and used for this purpose.

The invention also provides assays for detecting the presence of a STRAP protein in a tissue or other biological sample such as serum, bone, prostate, and other tissues, urine, cell preparations, and the like. Methods for detecting a STRAP protein are also well known 25 and include, for example, immunoprecipitation, immunohistochemical analysis, Western Blot analysis, molecular binding assays, ELISA, ELIFA and the like.

For example, in one embodiment, a method of detecting the presence of a STRAP protein in a biological sample comprises first contacting the sample with a STRAP antibody, a 30 STRAP-reactive fragment thereof, or a recombinant protein containing an antigen binding region of a STRAP antibody; and then detecting the binding of STRAP protein in the sample thereto.

Methods for identifying a cell which expresses STRAP are also provided. In one 35 embodiment, an assay for identifying a cell which expresses a STRAP gene comprises detecting the presence of STRAP mRNA in the cell. Methods for the detection of particular mRNAs in cells are well known and include, for example, hybridization assays using complementary DNA probes (such as *in situ* hybridization using labeled STRAP riboprobes, Northern blot and related techniques) and various nucleic acid amplification assays (such

as RT-PCR using complementary primers specific for STRAP, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like). Alternatively, an assay for identifying a cell which expresses a STRAP gene comprises detecting the presence of STRAP protein in the cell or secreted by the cell. Various 5 methods for the detection of proteins are well known in the art and may be employed for the detection of STRAP proteins and STRAP expressing cells.

STRAP expression analysis may also be useful as a tool for identifying and evaluating 10 agents which modulate STRAP gene expression. For example, STRAP-1 expression is significantly upregulated in colon, bladder, pancreatic, ovarian and other cancers. Identification of a molecule or biological agent that could inhibit STRAP-1 over-expression may be of therapeutic value in the treatment of cancer. Such an agent may be identified by using a screen that quantifies STRAP expression by RT-PCR, nucleic acid hybridization or antibody binding.

15

#### ASSAYS FOR DETERMINING STRAP EXPRESSION STATUS

Determining the status of STRAP expression patterns in an individual may be used to 20 diagnose cancer and may provide prognostic information useful in defining appropriate therapeutic options. Similarly, the expression status of STRAP may provide information useful for predicting susceptibility to particular disease stages, progression, and/or tumor aggressiveness. The invention provides methods and assays for determining STRAP expression status and diagnosing cancers which express STRAP.

In one aspect, the invention provides assays useful in determining the presence of 25 cancer in an individual, comprising detecting a significant increase in STRAP mRNA or protein expression in a test cell or tissue sample relative to expression levels in the corresponding normal cell or tissue. In one embodiment, the presence of STRAP-1 mRNA is evaluated in tissue samples of the colon, pancreas, bladder, ovary, cervix, testis or breast. The presence of significant STRAP-1 expression in any of these tissues may be 30 useful to indicate the emergence, presence and/or severity of these cancers, since the corresponding normal tissues do not express STRAP-1 mRNA. In a related embodiment, STRAP-1 expression status may be determined at the protein level rather than at the nucleic acid level. For example, such a method or assay would comprise determining the level of STRAP-1 protein expressed by cells in a test tissue sample and comparing the level 35 so determined to the level of STRAP expressed in a corresponding normal sample. In one embodiment, the presence of STRAP-1 protein is evaluated, for example, using immunohistochemical methods. STRAP antibodies or binding partners capable of detecting STRAP protein expression may be used in a variety of assay formats well known in the art for this purpose.

Peripheral blood may be conveniently assayed for the presence of cancer cells, including prostate, colon, pancreatic, bladder and ovarian cancers, using RT-PCR to detect STRAP-1 expression. The presence of RT-PCR amplifiable STRAP-1 mRNA provides an indication of the presence of one of these types of cancer. RT-PCR detection assays for tumor cells in peripheral blood are currently being evaluated for use in the diagnosis and management of a number of human solid tumors. In the prostate cancer field, these include RT-PCR assays for the detection of cells expressing PSA and PSM (Verkaik et al., 1997, Urol. Res. 25: 373-384; Ghossein et al., 1995, J. Clin. Oncol. 13: 1195-2000; Heston et al., 1995, Clin. Chem. 41: 1687-1688). RT-PCR assays are well known in the art.

In another approach, a recently described sensitive assay for detecting and characterizing carcinoma cells in blood may be used (Racila et al., 1998, Proc. Natl. Acad. Sci. USA 95: 4589-4594). This assay combines immunomagnetic enrichment with multiparameter flow cytometric and immunohistochemical analyses, and is highly sensitive for the detection of cancer cells in blood, reportedly capable of detecting one epithelial cell in 1 ml of peripheral blood.

A related aspect of the invention is directed to predicting susceptibility to developing cancer in an individual. In one embodiment, a method for predicting susceptibility to cancer comprises detecting STRAP mRNA or STRAP protein in a tissue sample, its presence indicating susceptibility to cancer, wherein the degree of STRAP mRNA expression present is proportional to the degree of susceptibility.

Yet another related aspect of the invention is directed to methods for gauging tumor aggressiveness. In one embodiment, a method for gauging aggressiveness of a tumor comprises determining the level of STRAP mRNA or STRAP protein expressed by cells in a sample of the tumor, comparing the level so determined to the level of STRAP mRNA or STRAP protein expressed in a corresponding normal tissue taken from the same individual or a normal tissue reference sample, wherein the degree of STRAP mRNA or STRAP protein expression in the tumor sample relative to the normal sample indicates the degree of aggressiveness.

Methods for detecting and quantifying the expression of STRAP mRNA or protein are described herein and use standard nucleic acid and protein detection and quantification technologies well known in the art. Standard methods for the detection and quantification of STRAP mRNA include *in situ* hybridization using labeled STRAP riboprobes, Northern blot and related techniques using STRAP polynucleotide probes, RT-PCR analysis using primers specific for STRAP, and other amplification type detection methods, such as, for

example, branched DNA, SISBA, TMA and the like. In a specific embodiment, semi-quantitative RT-PCR may be used to detect and quantify STRAP mRNA expression as described in the Examples which follow. Any number of primers capable of amplifying STRAP may be used for this purpose, including but not limited to the various primer sets 5 specifically described herein. Standard methods for the detection and quantification of protein may be used for this purpose. In a specific embodiment, polyclonal or monoclonal antibodies specifically reactive with the wild-type STRAP protein may be used in an immunohistochemical assay of biopsied tissue.

10 **DIAGNOSTIC IMAGING OF HUMAN CANCERS**

The expression profiles of STRAP-1 and STRAP-2 indicate antibodies specific therefor may be particularly useful in radionuclide and other forms of diagnostic imaging of certain cancers. For example, immunohistochemical analysis of STRAP-1 protein suggests that in normal tissues STRAP-1 is predominantly restricted to prostate and 15 bladder. The transmembrane orientation of STRAP-1 (and presumably STRAP-2) provides a target readily identifiable by antibodies specifically reactive with extracellular epitopes. This tissue restricted expression, and the localization of STRAP to the cell surface of multiple cancers makes STRAP an ideal candidate for diagnostic imaging. Accordingly, in vivo imaging techniques may be used to image human cancers 20 expressing a STRAP protein.

For example, cell surface STRAP-1 protein is expressed at very high levels in several human cancers, particularly prostate, bladder, colon and ovarian cancers, and Ewing sarcoma. Moreover, in normal tissues, STRAP-1 protein expression is largely restricted 25 to prostate. Thus, radiolabeled antibodies specifically reactive with extracellular epitopes of STRAP-1 may be particularly useful in in vivo imaging of solid tumors of the foregoing cancers. Such labeled anti-STRAP-1 antibodies may provide very high level sensitivities for the detection of metastasis of these cancers.

30 Preferably, monoclonal antibodies are used in the diagnostic imaging methods of the invention.

**CANCER IMMUNOTHERAPY AND CANCER VACCINES**

The invention provides various immunotherapeutic methods for treating prostate cancer, 35 including antibody therapy, in vivo vaccines, and ex vivo immunotherapy methods, which utilize polynucleotides and polypeptides corresponding to STRAP and STRAP antibodies. These therapeutic applications are described further in the following subsections.

Applicants have accumulated strong and compelling evidence that STRAP-1 is strongly expressed uniformly over the surface of glandular epithelial cells within prostate and prostate cancer cells. See, for details, immunohistochemical and Western blot analysis of STRAP-1 protein expression presented in Examples XX and XX as well as the STRAP-1 mRNA expression profiles obtained from the Northern blot and RT-PCR generated data presented in Examples XX and XX. In particular, immunohistochemical analysis results show that the surface of human prostate epithelial cells (normal and cancer) appear to be uniformly coated with STRAP-1. Biochemical analysis confirms the cell surface localization of STRAP-1 initially suggested by its putative 6-transmembrane primary structural elements and by the pericellular staining plainly visualized by immunohistochemical staining.

STRAP-1 is uniformly expressed at high levels over the surface of prostate glandular epithelia, an ideal situation for immunotherapeutic intervention strategies which target extracellular STRAP epitopes. Systemic administration of STRAP-immunoreactive compositions would be expected to result in extensive contact of the composition with prostate epithelial cells via binding to STRAP-1 extracellular epitopes. Moreover, given the near absence of STRAP-1 protein expression in normal human tissues, there is ample reason to expect exquisite sensitivity without toxic, non-specific and/or non-target effects caused by the binding of the immunotherapeutic composition to STRAP-1 on non-target organs and tissues.

In addition to the high level expression of STRAP-1 in prostate and prostate cancer cells, STRAP-1 appears to be substantially over-expressed in a variety of other human cancers, including bladder, colon, pancreatic and ovarian cancers. In particular, high level STRAP-1 mRNA expression is detected in all tested prostate cancer tissues and cell lines, and in most of the pancreatic, colon, and bladder cancer cell lines tested. High level expression of STRAP-1 is also observed in some ovarian cancer cell lines. Lower level expression is observed in some breast, testicular, and cervical cancer cell lines. Very high level expression is also detected in a Ewing sarcoma cell line. Applicants have shown that cell surface STRAP-1 protein is expressed in bladder and colon cancers, while there is no detectable cell surface (or intracellular) STRAP-1 protein in normal colon and low expression in normal bladder. Antibodies specifically reactive with extracellular domains of STRAP-1 may be useful to treat these cancers systemically, either as toxin or therapeutic agent conjugates or as naked antibodies capable of inhibiting cell proliferation or function.

STRAP-2 protein is also expressed in prostate cancer, and may be expressed in other cancers as well. STRAP-2 mRNA analysis by RT-PCR and Northern blot show that expression is restricted to prostate in normal tissues, is also expressed in some prostate, pancreatic, colon, testicular, ovarian and other cancers. Therefore, antibodies reactive

with STRAP-2 may be useful in the treatment of prostate and other cancers. Similarly, alth ugh not yet characterized by applicants, th expression of STRAP-3 and STRAP-4 (as well as other STRAPs) may be associated with some cancers. Thus antibodies reactive with these STRAP family member proteins may also be useful therapeutically.

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STRAP antibodies may be introduced into a patient such that the antibody binds to STRAP on the cancer cells and mediates the destruction of the cells and the tumor and/or inhibits the growth of the cells or the tumor. Mechanisms by which such antibodies exert a therapeutic effect may include complement-mediated cytolysis, antibody-dependent cellular cytotoxicity, modulating the physiologic function of STRAP, inhibiting ligand binding or signal transduction pathways, modulating tumor cell differentiation, altering tumor angiogenesis factor profiles, and/or by inducing apoptosis. STRAP antibodies conjugated to toxic or therapeutic agents may also be used therapeutically to deliver the toxic or therapeutic agent directly to STRAP-bearing tumor cells.

10

15 Cancer immunotherapy using anti-STRAP antibodies may follow the teachings generated from various approaches which have been successfully employed with respect to other types of cancer, including but not limited to colon cancer (Arlen et al., 1998, Crit Rev Immunol 18: 133-138), multiple myeloma (Ozaki et al., 1997, Blood 90: 3179-3186; Tsunenari et al., 1997, Blood 90: 2437-2444), gastric cancer (Kasprzyk et al., 1992, Cancer Res 52: 2771-2776), B-cell lymphoma (Funakoshi et al., 1996, J Immunther Emphasis Tumor Immunol 19: 93-101), leukemia (Zhong et al., 1996, Leuk Res 20: 581-589), colorectal cancer (Moun et al., 1994, Cancer Res 54: 6160-6166); Velders et al., 1995, Cancer Res 55: 4398-4403), and breast cancer (Shepard et al., 1991, J Clin Immunol 11: 117-127).

20

25 Although STRAP antibody therapy may be useful for all stages of the foregoing cancers, antibody therapy may be particularly appropriate and in advanced or metastatic cancers. Combining the antibody therapy method of the invention with a chemotherapeutic or radiation regimen may be preferred in patients who have not received chemotherapeutic 30 treatment, whereas treatment with the antibody therapy of the invention may be indicated for patients who have received one or more chemotherapy. Additionally, antibody therapy may also enable the use of reduced dosages of concomitant chemotherapy, particularly in patients that do not tolerate the toxicity of the chemotherapeutic agent very well.

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It may be desirable for non-prostate cancer patients to be evaluated for the presence and level of STRAP over-expression, preferably using immunohistochemical assessments of tumor tissue, quantitative STRAP imaging, or other techniques capable of reliably indicating th presence and degree of STRAP overexpression. Immunohistochemical

analysis of tumor biopsies or surgical specimens may be preferred for this purpose. Methods for immunohistochemical analysis of tumor tissues are well known in the art.

Anti-STRAP monoclonal antibodies useful in treating prostate and other cancers include 5 those which are capable of initiating a potent immune response against the tumor and those which are capable of direct cytotoxicity. In this regard, anti-STRAP mAbs may elicit tumor cell lysis by either complement-mediated or antibody-dependent cell cytotoxicity (ADCC) mechanisms, both of which require an intact Fc portion of the immunoglobulin molecule for interaction with effector cell Fc receptor sites or complement proteins. In 10 addition, anti-STRAP mAbs which exert a direct biological effect on tumor growth are useful in the practice of the invention. Potential mechanisms by which such directly cytotoxic mAbs may act include inhibition of cell growth, modulation of cellular differentiation, modulation of tumor angiogenesis factor profiles, and the induction of apoptosis. The mechanism by which a particular anti-STRAP mAb exerts an anti-tumor effect may be 15 evaluated using any number of in vitro assays designed to determine ADCC, ADMMC, complement-mediated cell lysis, and so forth, as is generally known in the art.

The anti-tumor activity of a particular anti-STRAP mAb, or combination of anti-STRAP mAbs, may be evaluated in vivo using a suitable animal model. For example, xenogenic 20 prostate cancer models wherein human prostate cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice, are appropriate in relation to prostate cancer and have been described (Klein et al., 1997, Nature Medicine 3: 402-408). For Example, PCT Patent Application WO98/16628, Sawyers et al., published April 23, 1998, describes various xenograft models of human prostate 25 cancer capable of recapitulating the development of primary tumors, micrometastasis, and the formation of osteoblastic metastases characteristic of late stage disease. Efficacy may be predicted using assays which measure inhibition of tumor formation, tumor regression or metastasis, and the like.

30 It should be noted that the use of murine or other non-human monoclonal antibodies, human/mouse chimeric mAbs may induce moderate to strong immune responses in some patients. In the most severe cases, such an immune response may lead to the extensive formation of immune complexes which, potentially, can cause renal failure. Accordingly, preferred monoclonal antibodies used in the practice of the therapeutic methods of the 35 invention are those which are either fully human or humanized and which bind specifically to the target 20P1F12/TMPRSS2 antigen with high affinity but exhibit low or no antigenicity in the patient.

The method of the invention contemplate the administration of single anti-STRAP mAbs as well as combinations, or "cocktails, of different mAbs. Such mAb cocktails may have certain advantages inasmuch as they contain mAbs which exploit different effector mechanisms or combine directly cytotoxic mAbs with mAbs that rely on immune effector functionality. Such mAbs in combination may exhibit synergistic therapeutic effects. In addition, the administration of anti-STRAP mAbs may be combined with other therapeutic agents, including but not limited to various chemotherapeutic agents, androgen-blockers, and immune modulators (e.g., IL-2, GM-CSF). The anti-STRAP mAbs may be administered in their "naked" or unconjugated form, or may have therapeutic agents conjugated to them.

10 The anti-STRAP monoclonal antibodies used in the practice of the method of the invention may be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material which when combined with the anti-STRAP mAbs retains the anti-tumor function of the antibody and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like.

15 The anti-STRAP antibody formulations may be administered via any route capable of delivering the antibodies to the tumor site. Potentially effective routes of administration include, but are not limited to, intravenous, intraperitoneal, intramuscular, intratumor, intradermal, and the like. The preferred route of administration is by intravenous injection. A preferred formulation for intravenous injection comprises the anti-STRAP mAbs in a solution of preserved bacteriostatic water, sterile unpreserved water, and/or 20 diluted in polyvinylchloride or polyethylene bags containing 0.9% sterile Sodium Chloride for Injection, USP. The anti-STRAP mAb preparation may be lyophilized and stored as a sterile powder, preferably under vacuum, and then reconstituted in bacteriostatic water containing, for example, benzyl alcohol preservative, or in sterile water prior to injection.

25 30 Treatment will generally involve the repeated administration of the anti-STRAP antibody preparation via an acceptable route of administration such as intravenous injection (IV), typically at a dose in the range of about 0.1 to about 10 mg/kg body weight. Doses in the range of 10-500 mg mAb per week may be effective and well tolerated. Based on clinical experience with the Herceptin mAb in the treatment of metastatic breast cancer, an initial loading dose of approximately 4 mg/kg patient body weight IV followed by weekly doses of about 2 mg/kg IV of the anti-STRAP mAb preparation may represent an acceptable dosing regimen. Preferably, the initial loading dose is administered as a 90 minute or longer infusion. The periodic maintenance dose may be administered as a 30 minute or longer infusion, provided the initial dose was well tolerated. However, as one of skill in the art will

35

understand, various factors will influence the ideal dose regimen in a particular case. Such factors may include, for example, the binding affinity and half life of the mAb or mAbs used, the degree of STRAP overexpression in the patient, the extent of circulating shed STRAP antigen, the desired steady-state antibody concentration level, frequency of treatment, and 5 the influence of chemotherapeutic agents used in combination with the treatment method of the invention.

Optimally, patients should be evaluated for the level of circulating shed STRAP antigen in 10 serum in order to assist in the determination of the most effective dosing regimen and related factors. Such evaluations may also be used for monitoring purposes throughout therapy, and may be useful to gauge therapeutic success in combination with evaluating other parameters (such as serum PSA levels in prostate cancer therapy).

#### CANCER VACCINES

15 The invention further provides prostate cancer vaccines comprising a STRAP protein or fragment thereof. The use of a tumor antigen in a vaccine for generating humoral and cell-mediated immunity for use in anti-cancer therapy is well known in the art and has been employed in prostate cancer using human PSMA and rodent PAP immunogens (Hodge et al., 1995, Int. J. Cancer 63: 231-237; Fong et al., 1997, J. Immunol. 159: 3113-3117). Such 20 methods can be readily practiced by employing a STRAP protein, or fragment thereof, or a STRAP-encoding nucleic acid molecule and recombinant vectors capable of expressing and appropriately presenting the STRAP immunogen.

25 For example, viral gene delivery systems may be used to deliver a STRAP-encoding nucleic acid molecule. Various viral gene delivery systems which can be used in the practice of this aspect of the invention include, but are not limited to, vaccinia, fowlpox, canarypox, adenovirus, influenza, poliovirus, adeno-associated virus, lentivirus, and sindbus virus (Restifo, 1996, Curr. Opin. Immunol. 8: 658-663). Non-viral delivery systems may also be employed by using naked DNA encoding a STRAP protein or fragment thereof introduced 30 into the patient (e.g., intramuscularly) to induce an anti-tumor response. In one embodiment, the full-length human STRAP cDNA may be employed. In another embodiment, STRAP nucleic acid molecules encoding specific cytotoxic T lymphocyte (CTL) epitopes may be employed. CTL epitopes can be determined using specific algorithms (e.g., Epimer, Brown University) to identify peptides within a STRAP protein 35 which are capable of optimally binding to specified HLA alleles.

Various ex vivo strategies may also be employed. One approach involves the use of dendritic cells to present STRAP antigen to a patient's immune system. Dendritic cells express MHC class I and II, B7 costimulator, and IL-12, and are thus highly specialized

antigen presenting cells. In prostate cancer, autologous dendritic cells pulsed with peptides of the prostate-specific membrane antigen (PSMA) are being used in a Phase I-clinical trial to stimulate prostate cancer patients' immun systems (Tjoa et al., 1996, *Prostate* 28: 65-69; Murphy et al., 1996, *Prostate* 29: 371-380). Dendritic cells can be  
5 used to present STRAP peptides to T cells in the context of MHC class I and II molecules. In one embodiment, autologous dendritic cells are pulsed with STRAP peptides capable of binding to MHC molecules. In another embodiment, dendritic cells are pulsed with the complete STRAP protein. Yet another embodiment involves engineering the overexpression of the STRAP gene in dendritic cells using various implementing vectors  
10 known in the art, such as adenovirus (Arthur et al., 1997, *Cancer Gene Ther.* 4: 17-25), retrovirus (Henderson et al., 1996, *Cancer Res.* 56: 3763-3770), lentivirus, adeno-associated virus, DNA transfection (Ribas et al., 1997, *Cancer Res.* 57: 2865-2869), and tumor-derived RNA transfection (Ashley et al., 1997, *J. Exp. Med.* 186: 1177-1182).

15 Anti-idiotypic anti-STRAP antibodies can also be used in anti-cancer therapy as a vaccine for inducing an immune response to cells expressing a STRAP protein. Specifically, the generation of anti-idiotypic antibodies is well known in the art and can readily be adapted to generate anti-idiotypic anti-STRAP antibodies that mimic an epitope on a STRAP protein (see, for example, Wagner et al., 1997, *Hybridoma* 16: 33-40; Foon et al., 1995, *J Clin Invest* 96: 334-342; Herlyn et al., 1996, *Cancer Immunol Immunother* 43: 65-76). Such an anti-idiotypic antibody can be used in anti-idiotypic therapy as presently practiced with other anti-idiotypic antibodies directed against tumor antigens.

20 Genetic immunization methods may be employed to generate prophylactic or therapeutic humoral and cellular immune responses directed against cancer cells expressing STRAP. Constructs comprising DNA encoding a STRAP protein/immunogen and appropriate regulatory sequences may be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take-up the construct and express the encoded STRAP protein/immunogen. Expression of the STRAP protein immunogen results in the generation  
25 of prophylactic or therapeutic humoral and cellular immunity against prostate cancer. Various prophylactic and therapeutic genetic immunization techniques known in the art may be used (for review, see information and references published at Internet address [www.genweb.com](http://www.genweb.com)).

30 **35 KITS**  
For use in the diagnostic and therapeutic applications described or suggested above, kits are also provided by the invention. Such kits may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes, and the like, each of the container means comprising one of the separate

elements to be used in the method. For example, one of the container means may comprise a probe which is or can be detectably labeled. Such probe may be an antibody- or polynucleotide specific for a STRAP protein or a STRAP gene or message, respectively. Where the kit utilizes nucleic acid hybridization to detect the target nucleic acid, the kit may also have containers containing nucleotide(s) for amplification of the target nucleic acid sequence and/or a container comprising a reporter-means, such as a biotin-binding protein, such as avidin or streptavidin, bound to a reporter molecule, such as an enzymatic, fluorescent, or radionucleotide label.

10

#### EXAMPLES

Various aspects of the invention are further described and illustrated by way of the several examples which follow, none of which are intended to limit the scope of the 15 invention.

##### EXAMPLE 1:

##### ISOLATION OF cDNA FRAGMENT OF STRAP-1 GENE

20 MATERIALS AND METHODS

###### Cell lines and Human Tissues

All human cancer cell lines used in this study were obtained from the ATCC. All cell lines were maintained in DMEM with 10% fetal calf serum. PrEC (primary prostate epithelial 25 cells) were obtained from Clonetics and were grown in PrEBM media supplemented with growth factors (Clonetics).

All human prostate cancer xenografts were originally provided by Charles Sawyers (UCLA) (Klein et al., 1997). LAPC-4 AD and LAPC-9 AD xenografts were routinely 30 passaged as small tissue chunks in recipient SCID males. LAPC-4 AI and LAPC-9 AI xenografts were derived as described previously (Klein et al., 1997) and were passaged in castrated males or in female SCID mice. A benign prostatic hyperplasia tissue sample was patient-derived.

35 Human tissues for RNA and protein analyses were obtained from the Human Tissue Resource Center (HTRC) at the UCLA (Los Angeles, CA) and from QualTek, Inc. (Santa Barbara, CA).

RNA Isolation:

Tumor tissue and cell lines were homogenized in Trizol reagent (Life Technologies, Gibco BRL) using 10 ml/ g tissue or 10 ml/ 10<sup>6</sup> cells to isolate total RNA. Poly A RNA was purified from total RNA using Qiagen's Oligotex mRNA Mini and Midi kits. Total and mRNA were 5 quantified by spectrophotometric analysis (O.D. 260/280 nm) and analyzed by gel electrophoresis.

Oligonucleotides:

The following HPLC purified oligonucleotides were used.

10

RSACDN (cDNA synthesis primer):

5'TTTTGTACAAGCTT<sub>30</sub>3'

15

Adaptor 1:

5'CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCGGCAGGT3'  
3'GGCCCGTCCA5'

Adaptor 2:

5'GTAATACGACTCACTATAGGGCAGCGTGGTCGCCGGCGAGGT3'  
3'CGGCTCCA5'

20

PCR primer 1:

5'CTAATACGACTCACTATAGGGC3'

Nested primer (NP)1:

5'TCGAGCGGCCGCCGGCAGGT3'

25

Nested primer (NP)2:

5'AGCGTGGTCGCCGGCGAGGT3'

Suppression Subtractive Hybridization:

30 Suppression Subtractive Hybridization (SSH) was used to identify cDNAs corresponding to genes which may be up-regulated in androgen dependent prostate cancer compared to benign prostatic hyperplasia.

35 Double stranded cDNAs corresponding to the LAPC-4 AD xenograft (tester) and the BPH tissue (driver) were synthesized from 2 µg of poly(A)<sup>+</sup> RNA isolated from xenograft and BPH tissue, as described above, using CLONTECH's PCR-Select cDNA Subtraction Kit and 1 ng of oligonucleotide RSACDN as primer. First- and second-strand synthesis were carried out as described in the Kit's user manual protocol (CLONTECH Protocol No. PT1117-1, Catalog No. K1804-1). The resulting cDNA was digested with Rsa I for 3 hrs. at

37°C. Digested cDNA was extracted with phenol/chloroform (1:1) and ethanol precipitated.

5 Driver cDNA (BPH) was generated by combining in a 4 to 1 ratio Rsa I digested BPH cDNA with digested cDNA from mouse liver, in order to ensure that murine genes were subtracted from the tester cDNA (LAPC-4 AD).

10 Tester cDNA (LAPC-4 AD) was generated by diluting 1  $\mu$ l of Rsa I digested LAPC-4 AD cDNA (400 ng) in 5  $\mu$ l of water. The diluted cDNA (2  $\mu$ l, 160 ng) was then ligated to 2  $\mu$ l of adaptor 1 and adaptor 2 (10  $\mu$ M), in separate ligation reactions, in a total volume of 10  $\mu$ l at 16°C overnight, using 400  $\mu$ u of T4 DNA ligase (CLONTECH). Ligation was terminated with 1  $\mu$ l of 0.2 M EDTA and heating at 72°C for 5 min.

15 The first hybridization was performed by adding 1.5  $\mu$ l (600 ng) of driver cDNA to each of two tubes containing 1.5  $\mu$ l (20 ng) adaptor 1- and adaptor 2- ligated tester cDNA. In a final volume of 4  $\mu$ l, the samples were overlayed with mineral oil, denatured in an MJ Research thermal cycler at 98°C for 1.5 minutes, and then were allowed to hybridize for 8 hrs at 68°C. The two hybridizations were then mixed together with an additional 1  $\mu$ l of fresh denatured driver cDNA and were allowed to hybridize overnight at 68°C. The 20 second hybridization was then diluted in 200  $\mu$ l of 20 mM Hepes, pH 8.3, 50 mM NaCl, 0.2 mM EDTA, heated at 70°C for 7 min. and stored at -20°C.

PCR Amplification, Cloning and Sequencing of Gene Fragments Generated from SSH:  
To amplify gene fragments resulting from SSH reactions, two PCR amplifications were 25 performed. In the primary PCR reaction 1  $\mu$ l of the diluted final hybridization mix was added to 1  $\mu$ l of PCR primer 1 (10  $\mu$ M), 0.5  $\mu$ l dNTP mix (10  $\mu$ M), 2.5  $\mu$ l 10 x reaction buffer (CLONTECH) and 0.5  $\mu$ l 50 x Advantage cDNA polymerase Mix (CLONTECH) in a final volume of 25  $\mu$ l. PCR 1 was conducted using the following conditions: 75°C for 5 min., 94°C for 25 sec., then 27 cycles of 94°C for 10 sec, 66°C for 30 sec, 72°C for 1.5 min. Five 30 separate primary PCR reactions were performed for each experiment. The products were pooled and diluted 1:10 with water. For the secondary PCR reaction, 1  $\mu$ l from the pooled and diluted primary PCR reaction was added to the same reaction mix as used for PCR 1, except that primers NP1 and NP2 (10  $\mu$ M) were used instead of PCR primer 1. PCR 2 was performed using 10-12 cycles of 94°C for 10 sec, 68°C for 30 sec, 72°C for 1.5 35 minutes. The PCR products were analyzed using 2% agarose gel electrophoresis.

The PCR products were inserted into pCR2.1 using the T/A vector cloning kit (Invitrogen). Transformed *E. coli* were subjected to blue/white and ampicillin selection. White colonies

were picked and arrayed into 96 well plates and were grown in liquid culture overnight. To identify inserts, PCR amplification was performed on 1 ml of bacterial culture using the conditions of PCR1 and NP1 and NP2 as primers. PCR products were analyzed using 2% agarose gel electrophoresis.

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Bacterial clones were stored in 20% glycerol in a 96 well format. Plasmid DNA was prepared, sequenced, and subjected to nucleic acid homology searches of the GenBank, dBest, and NCI-CGAP databases.

10 **RT-PCR Expression Analysis:**

First strand cDNAs were generated from 1  $\mu$ g of mRNA with oligo (dT)12-18 priming using the Gibco-BRL Superscript Preamplification system. The manufacturers protocol was used and included an incubation for 50 min at 42°C with reverse transcriptase followed by RNase H treatment at 37°C for 20 min. After completing the reaction, the volume was 15 increased to 200  $\mu$ l with water prior to normalization. First strand cDNAs from 16 different normal human tissues were obtained from Clontech.

20 Normalization of the first strand cDNAs from multiple tissues was performed by using the primers 5'atatcgccgcgtcgatcgacaa3' and 5'agccacacgcagctcattgttagaagg 3' to amplify  $\beta$ -actin. First strand cDNA (5  $\mu$ l) was amplified in a total volume of 50  $\mu$ l containing 0.4  $\mu$ M primers, 0.2  $\mu$ M each dNTPs, 1XPCR buffer (Clontech, 10 mM Tris-HCL, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, pH8.3) and 1X KlenTaq DNA polymerase (Clontech). Five  $\mu$ l of the PCR reaction was removed at 18, 20, and 22 cycles and used for agarose gel electrophoresis. 25 PCR was performed using an MJ Research thermal cycler under the following conditions: initial denaturation was at 94°C for 15 sec, followed by 18, 20, and 22 cycles of 94°C for 15, 65°C for 2 min, 72°C for 5 sec. A final extension at 72°C was carried out for 2 min. After agarose gel electrophoresis, the band intensities of the 283 bp  $\beta$ -actin bands from 30 multiple tissues were compared by visual inspection. Dilution factors for the first strand cDNAs were calculated to result in equal  $\beta$ -actin band intensities in all tissues after 22 cycles of PCR. Three rounds of normalization were required to achieve equal band intensities in all tissues after 22 cycles of PCR.

35 To determine expression levels of the 8P1D4 gene, 5  $\mu$ l of normalized first strand cDNA was analyzed by PCR using 25, 30, and 35 cycles of amplification using the following primer pairs, which were designed with the assistance of (MIT; for details, see, [www.genome.wi.mit.edu](http://www.genome.wi.mit.edu)):

5' ACT TTG TTG ATG ACC AGG ATT GGA 3'  
5' CAG AAC TTC AGC ACA CAC AGG AAC 3'

Semi quantitative expression analysis was achieved by comparing the PCR products at cycle numbers that give light band intensities.

5

**RESULTS:**

Several SSH experiments were conducted as described in the Materials and Methods, supra, and led to the isolation of numerous candidate gene fragment clones. All candidate clones were sequenced and subjected to homology analysis against all 10 sequences in the major public gene and EST databases in order to provide information on the identity of the corresponding gene and to help guide the decision to analyze a particular gene for differential expression. In general, gene fragments which had no homology to any known sequence in any of the searched databases, and thus considered to represent novel genes, as well as gene fragments showing homology to previously 15 sequenced expressed sequence tags (ESTs), were subjected to differential expression analysis by RT-PCR and/or Northern analysis.

One of the cDNA clones, designated 8P1D4, was 436 bp in length and showed homology to an EST sequence in the NCI-CGAP tumor gene database. The full length cDNA 20 encoding the 8P1D4 gene was subsequently isolated using this cDNA and re-named STRAP-1 (Example XX, below). The 8P1D4 cDNA nucleotide sequence corresponds to nucleotide residues 150 through 585 in the STRAP-1 cDNA sequence as shown in FIG. 1A. Another clone, designated 28P3E1, 561 bp in length showed homology to a number 25 of EST sequences in the NCI-CGAP tumor gene database or in other databases. Part of the 28P3E1 sequence (356 bp) is identical to an EST derived from human fetal tissue. After the full length STRAP-1 cDNA was obtained and sequenced, it became apparent that this clone also corresponds to STRAP-1 (more specifically, to residues 622 through the 3' end of the STRAP-1 nucleotide sequence as shown in FIG. 1A).

30 Differential expression analysis by RT-PCR using primers derived from the 8P1D4 cDNA clone showed that the 8P1D4 (STRAP-1) gene is expressed at approximately equal levels in normal prostate and the LAPC-4 and LAPC-9 xenografts (FIG. 2, panel A). Further RT-PCR expression analysis of first strand cDNAs from 16 normal tissues showed greatest 35 levels of 8P1D4 expression in prostate. Substantially lower level expression in several other normal tissues (i.e., colon, ovary, small intestine, spleen and testis) was detectable only at 30 cycles of amplification in brain, pancreas, colon and small intestine (FIG. 2, panels B and C).

**EXAMPLE 2:****ISOLATION OF FULL LENGTH STRAP-1 ENCODING cDNA**

The 436 bp 8P1D4 gene fragment (Example 1) was used to isolate additional cDNAs encoding the 8P1D4/STRAP-1 gene. Briefly, a normal human prostate 5 cDNA library (Clontech) was screened with a labeled probe generated from the 436 bp 8P1D4 cDNA. One of the positive clones, clone 10, is 1195 bp in length and encodes a 339 amino acid protein having nucleotide and encoded amino acid sequences bearing no significant homology to any known human genes or 10 proteins (homology to a rat Kidney Injury Protein recently described in International Application WO98/53071). The encoded protein contains at least 6 predicted transmembrane motifs implying a cell surface orientation (see FIG. 1A, predicted transmembrane motifs underlined). These structural features led to the 15 designation "STRAP", for "Serpentine TRansmembrane Antigen of the Prostate". Subsequent identification of additional STRAP proteins led to the re-designation 20 of the 8P1D4 gene product as "STRAP-1". The STRAP-1 cDNA and encoded amino acid sequences are shown in FIG. 1A and correspond to SEQ ID NOS: XX and XX, respectively. STRAP-1 cDNA clone 10 has been deposited with the American Type Culture Collection ("ATCC") (Mannassas, VA) as plasmid 8P1D4 clone 10.1 on August 26, 1998 as ATCC Accession Number 98849. The STRAP-1 cDNA clone can be excised therefrom using EcoRI/XbaI double digest (EcoRI at the 5'end, XbaI at the 3'end).

**EXAMPLE 3:****STRAP-1 GENE AND PROTEIN EXPRESSION ANALYSIS**

25 In order to begin to characterize the biological characteristics of STRAP-1, an extensive evaluation of STRAP-1 mRNA and STRAP-1 protein expression across a variety of human tissue specimens was undertaken. This evaluation included Northern blot, Western blot and immunohistochemical analysis of STRAP-1 expression in a large number of normal human tissues, human prostate cancer 30 xenografts and cell lines, and various other human cancer cell lines.

**Example 3A: Northern Blot Analysis of STRAP-1 mRNA Expression in Normal Human Tissues**

Initial analysis of STRAP-1 mRNA expression in normal human tissues was conducted by 35 Northern blotting two multiple tissue blots obtained from Clontech (Palo Alto, California), comprising a total of 16 different normal human tissues, using labeled STRAP-1 clone 10 as a probe. RNA samples were quantitatively normalized with a  $\beta$ -actin probe. The results are shown in FIG. 3A. The highest expression level was detected in normal prostate, with an approximately 5-10 fold lower level of expression detected in colon and

liver. These northern blots showed two transcripts of approximately 1.4 kb and 4.0 kb, the former of which corresponds to the full length STRAP-1 clone 10 cDNA, which encodes the entire STRAP-1 open reading frame. The larger transcript was separately cloned as a 3627 bp cDNA from a normal prostate library, the sequence of which 5 contains a 2399 bp intron (FIG. 4).

This initial analysis was extended by using the STRAP-1 clone 10 probe to analyze an RNA dot blot matrix of 37 normal human tissues (Clontech, Palo Alto, CA; Human Master Blot™). The results are shown in FIG. 3B and show strong STRAP-1 expression only in 10 prostate. Very low level STRAP-1 RNA expression was detected in liver, lung, trachea and fetal liver tissue, at perhaps a 5-fold lower level compared to prostate. No expression was detected in any of the remaining tissues. Based on these analyses, significant STRAP-1 expression appears to be prostate specific in normal tissues.

15 **Example 3B: Northern Blot Analysis of STRAP-1 mRNA Expression in Prostate Cancer Xenografts and Cell Lines**

To analyze STRAP-1 expression in human cancer tissues and cell lines, RNAs derived from human prostate cancer xenografts and an extensive panel of prostate and non-prostate cancer cell lines were analyzed by Northern blot using STRAP-1 cDNA clone 10 20 as probe. All RNA samples were quantitatively normalized by ethidium bromide staining and subsequent analysis with a labeled β-actin probe.

The results, presented in FIG. 5, show high level STRAP-1 expression in all the LAPC 25 xenografts and all of the prostate cancer cell lines. Expression in the LAPC-9 xenografts was higher compared to the LAPC-4 xenografts, with no significant difference observed between androgen-dependent and androgen-independent sublines (FIG. 5A). Expression in the LAPC-4 xenografts was comparable to expression in normal prostate. Lower levels of expression were detected in PrEC cells (Clonetics), which represent the basal cell 30 compartment of the prostate. Analysis of prostate cancer cell lines showed highest expression levels in LNCaP, an androgen dependent prostate carcinoma cell line. Significant expression was also detected in the androgen-independent cell lines PC-3 and DU145. High levels of STRAP expression were also detected in LAPC-4 and LAPC-9 tumors that were grown within the tibia of mice as a model of prostate cancer bone metastasis (FIG. 5B).

35 Significantly, very strong STRAP-1 expression was also detected in many of the non-prostate human cancer cell lines analyzed (FIG. 5A). Particularly high level expression was observed in RD-ES cells, an Ewing sarcoma (EWS) derived cell line. Additionally, very high level expression was also detected in several of the colon cancer cell lines

(e.g., CaCo-2, LoVo, T84 and Colo-205), bladder carcinoma cell lines (e.g., SCABER, UM-UC-3, TCCSUP and 5637), ovarian cancer cell lines (e.g., OV-1063 and SW 626) and pancreatic cancer cell lines (e.g., HPAC, Capan-1, PANC-1 and BxPC-3). These results, combined with the absence of strong expression in the corresponding normal tissues 5 (FIG. 3), indicate that STRAP-1 may be generally up-regulated in these types (as well as other types) of human cancers.

Example 3C: Western Blot Analysis of STRAP-1 Protein Expression in Prostate and Other Cancers

10 A 15 mer peptide corresponding to amino acid residues 14 through 28 of the STRAP-1 amino acid sequence as shown in FIG. 1A (WKMKP RRNLE EDDYL)(SEQ ID NO: XX) was synthesized and used to immunize sheep for the generation of sheep polyclonal antibodies towards the amino-terminus of the protein (anti-STRAP-1) as follows. The peptide was conjugated to KLH (keyhole limpet hemocyanin). The sheep was initially 15 immunized with 400 µg of peptide in complete Freund's adjuvant. The animal was subsequently boosted every two weeks with 200 µg of peptide in incomplete Freund's adjuvant. Anti-STRAP antibody was affinity-purified from sheep serum using STRAP peptide coupled to affi-gel 10 (Bio Rad). Purified antibody is stored in phosphate-buffered saline with 0.1% sodium azide.

20 To test antibody specificity, the cDNA of STRAP-1 was cloned into a retroviral expression vector (pSR $\alpha$ tkneo, Muller et al., 1991, MCB 11:1785). NIH 3T3 cells were infected with retroviruses encoding STRAP-1 and were selected in G418 for 2 weeks. Western blot analysis of protein extracts of infected and un-infected NIH 3T3 cells showed expression 25 of a protein with an apparent molecular weight of 36 kD only in the infected cells (FIG. 6, lanes marked "3T3 STRAP" AND "3T3").

30 The anti-STRAP-1 polyclonal antibody was used to probe Western blots of cell lysates prepared from a variety of prostate cancer xenograft tissues, prostate cancer cell lines and other non-prostate cancer cell lines. Protein samples (20 $\mu$ g each) were quantitatively normalized by probing the blots with an anti-Grb-2 antibody.

35 The results are shown in FIG. 6. STRAP-1 protein was detected in all of the LAPC prostate cancer xenografts, all of the prostate cancer cell lines, a primary prostate cancer specimen and its matched normal prostate control. Highest STRAP-1 protein expression was detected in the LAPC-9 xenograft and in LNCaP cells, in agreement with the Northern blot analysis described immediately above. High level expression was also observed in the bladder carcinoma cell line UM-UC-3. Expression in other cancer cell lines was also detectabl (FIG. 6).

Example 3D: Immunohistochemical Analysis of STRAP-1 Protein Expression in -  
Pr state Tumor Biopsy and Surgical Specimens

To determine the extent of STRAP-1 protein expression in clinical materials, tissue  
5 sections were prepared from a variety of prostate cancer biopsies and surgical samples  
for immunohistochemical analysis. Tissues were fixed in 10% formalin, embedded in  
paraffin, and sectioned according to standard protocol. Formalin-fixed, paraffin-  
embedded sections of LNCaP cells were used as a positive control. Sections were  
stained with an anti-STRAP-1 polyclonal antibody directed against a STRAP-1 N-terminal  
10 epitope (as described immediately above). LNCaP sections were stained in the presence  
of an excess amount of the STRAP-1 N-terminal peptide immunogen used to generate the  
polyclonal antibody (peptide 1) or a non-specific peptide derived from a distinct region of  
the STRAP-1 protein (peptide 2; YQQVQQNKEDAWIEH).

15 The results are shown in FIG. 8. LNCaP cells showed uniformly strong peri-cellular  
staining in all cells (FIG. 8b). Excess STRAP N-terminal peptide (peptide 1) was able to  
competitively inhibit antibody staining (FIG. 8a), while peptide 2 had no effect (FIG. 8b).  
Similarly, uniformly strong peri-cellular staining was seen in the LAPC-9 (FIG. 8f) and  
20 LAPC-4 prostate cancer xenografts (data not shown). These results are clear and  
suggest that the staining is STRAP specific. Moreover, these results visually localize  
STRAP to the plasma membrane, corroborating the biochemical findings presented in  
Example 4 below.

25 The results obtained with the various clinical specimens are show in FIG. 8c (normal  
prostate tissue), FIG. 8d (grade 3 prostatic carcinoma), and FIG. 8e (grade 4 prostatic  
carcinoma), and are also included in the summarized results shown in TABLE 1. Light to  
strong staining was observed in the glandular epithelia of all prostate cancer samples  
tested as well as in all samples derived from normal prostate or benign disease. The  
30 signal appears to be strongest at the cell membrane of the epithelial cells, especially at  
the cell-cell junctions (FIG. 8c, d and e) and is also inhibited with excess STRAP N-  
terminal peptide 1 (data not shown). Some basal cell staining is also seen in normal  
prostate (FIG. 8c), which is more apparent when examining atrophic glands (data not  
shown). STRAP-1 seems to be expressed at all stages of prostate cancer since lower  
35 grades (FIG. 8d), higher grades (FIG. 8e) and metastatic prostate cancer (represented by  
LAPC-9; FIG. 8f) all exhibit strong staining.

Immunohistochemical staining of a large panel of normal non-prostate tissues showed no  
detectable STRAP-1 expression in 24 of 27 of these normal tissues (Table 1). Only three  
tissue samples showed some degree of anti-STRAP-1 staining. In particular, normal

bladder exhibited low levels of cell surface staining in the transitional epithelium (FIG. 8g). Pancreas and pituitary showed low levels of cytoplasmic staining (Table 1). It is unclear whether the observed cytoplasmic staining is specific or is due to non-specific binding of the antibody, since northern blotting showed little to no STRAP-1 expression in pancreas (FIG. 3). Normal colon, which exhibited higher mRNA levels than pancreas by Northern blotting (FIG. 3), exhibited no detectable staining with anti-STRAP antibodies (FIG. 8h). These results indicate that cell surface expression of STRAP-1 in normal tissues appears to be restricted to prostate and bladder.

10

TABLE 1: IMMUNOHISTOCHEMICAL STAINING OF HUMAN  
TISSUES WITH ANTI-STRAP-1 POLYCLONAL ANTIBODY

	STAINING INTENSITY	TISSUE
15	NONE	cerebellum, cerebral cortex, spinal cord, heart, skeletal muscle, artery, thymus, spleen, bone marrow, lymph node, lung, colon, liver, stomach, kidney, testis, ovary, fallopian tubes, placenta, uterus, breast, adrenal gland, thyroid gland, skin, bladder (3/5)
20	LIGHT TO MODERATE	bladder (2/5), pituitary gland (cytoplasmic), pancreas (cytoplasmic), BPH (3/5), prostate cancer (3/10)
25	STRONG	prostate (2/2), BPH (2/5), prostate cancer** (7/10)

\*In cases where more than one sample is analyzed per tissue, the numbers in brackets indicates how many samples correspond to the staining category/total analyzed.

\*\*Prostate cancer grades ranged from Gleason grades 3 to 5.

30

EXAMPLE 4:

BIOCHEMICAL CHARACTERIZATION OF STRAP-1 PROTEIN

To initially characterize the STRAP-1 protein, cDNA clone 10 (SEQ ID NO. XX) was cloned 35 into the pcDNA 3.1 Myc-His plasmid (Invitrogen), which encodes a 6His tag at the carboxyl-terminus, transfected into 293T cells, and analyzed by flow cytometry using anti-His monoclonal antibody (His-probe, Santa Cruz) as well as the anti-STRAP-1 polyclonal antibody described above. Staining of cells was performed on intact cells as

well as permeabilized cells. The results indicated that only permeabilized cells stained with both antibodies, suggesting that both termini of the STRAP-1 protein are localized intracellularly. It is therefore possible that one or more of the STRAP-1 protein termini are associated with intracellular organelles rather than the plasma membrane.

5

To determine whether STRAP-1 protein is expressed at the cell surface, intact STRAP-1-transfected 293T cells were labeled with a biotinylation reagent that does not enter live cells. STRAP-1 was then immunoprecipitated from cell extracts using the anti-His and anti-STRAP antibodies. SV40 large T antigen, an intracellular protein that is expressed at high levels in 293T cells, and the endogenous cell surface transferrin receptor were immunoprecipitated as negative and positive controls, respectively. After immunoprecipitation, the proteins were transferred to a membrane and visualized with horseradish peroxidase-conjugated streptavidin. The results of this analysis are shown in FIG. 7. Only the transferrin receptor (positive control) and STRAP-1 were labeled with biotin, while the SV40 large T antigen (negative control) was not detectably labeled (FIG. 7A). Since only cell surface proteins are labeled with this technique, it is clear from these results that STRAP-1 is a cell surface protein. Combined with the results obtained from the flow cytometric analysis, it is clear that STRAP-1 is a cell surface protein with intracellular amino- and carboxyl- termini.

20

Furthermore, the above results together with the STRAP-1 secondary structural predictions, shows that STRAP-1 is a type IIIa membrane protein with a molecular topology of six potential transmembrane domains, 3 extracellular loops, 2 intracellular loops and two intracellular termini. A schematic representation of STRAP-1 protein topology relative to the cell membrane is shown in FIG. 1B.

25

In addition, prostate, bladder and colon cancer cells were directly analyzed for cell surface expression of STRAP-1 by biotinylation studies. Briefly, biotinylated cell surface proteins were affinity purified with streptavidin-gel and probed with the anti-STRAP-1 polyclonal antibody described above. Western blotting of the streptavidin purified proteins clearly show cell surface biotinylation of endogenous STRAP-1 in all prostate (LNCaP, PC-3, DU145), bladder (UM-UC-3, TCCSUP) and colon cancer (LoVo, Colo) cells tested, as well as in NIH 3T3 cells infected with a STRAP-1 encoding retrovirus, but not in non-expressing NIH 3T3 cells used as a negative control (FIG. 7B). In a further negative control, STRAP-1 protein was not detected in streptavidin precipitates from non-biotinylated STRAP expressing cells (FIG. 7B).

**EXAMPLE 5:****IDENTIFICATION AND STRUCTURAL ANALYSIS OF STRAP-2  
AND OTHER HUMAN STRAP FAMILY MEMBERS**

STRAP-1 has no homology to any known human genes. In an attempt to identify  
5 additional genes that are homologous to STRAP-1, the protein sequence of STRAP-1 was  
used as an electronic probe to identify family members in the public EST (expression  
sequence tag) database (dbest). Using the "tblastn" function in NCBI (National Center  
for Biotechnology Information), the dbest database was queried with the STRAP-1  
protein sequence. This analysis revealed additional putative STRAP-1 homologues or  
10 STRAP family members, as further described below.

In addition, applicants cloning experiments also identified a STRAP-1 related SSH cDNA  
fragment, clone 98P4B6. This clone was isolated from SSH cloning using normal  
prostate cDNA as tester and L APC-4 AD cDNA as driver. A larger partial sequence of the  
15 98P4B6 clone was subsequently isolated from a normal prostate library; this clone  
encodes an ORF of 173 amino acids with close homology to the primary structure of  
STRAP-1, and thus was designated STRAP-2.

The STRAP-2 partial nucleotide and encoded ORF amino acid sequences are shown in  
20 FIG. 9. An amino acid alignment of the STRAP-1 and partial STRAP-2 primary structures  
is shown in FIG. 11A. STRAP-1 and -2 share 61% identity over their 171 amino acid  
residue overlap (FIG. 11A). Despite their homology, STRAP-1 and -2 show significantly  
divergent expression patterns in normal and cancerous tissues and cells, and also map  
25 to distinct locations on opposite arms of human chromosome 7 (see Examples XX and XX  
below).

Two ESTs identified by electronic probing with the STRAP-1 protein sequence, AI139607  
and R80991, encode ORFs bearing close homology to the STRAP-1 and STRAP-2  
30 sequences and thus appear to represent two additional STRAPs. Their nucleotide  
sequences are reproduced in FIG. 10 and their encoded ORF STRAP-like amino acid  
sequences are shown in FIG. 11B. The ORFs encoded by these ESTs are unique but  
show very clear structural relationships to both STRAP-1 and STRAP-2, particularly in the  
conserved transmembrane domains. Accordingly these ESTs appear to correspond to  
35 distinct STRAP family members and have thus been designated as STRAP-3  
(corresponding to AI139607) and STRAP-4 (corresponding to R80991).

An amino acid alignment of the complete STRAP-1 protein sequence with the predicted  
partial STRAP-2, STRAP-3 and STRAP-4 amino acid sequences is shown in FIG. 11B.  
This alignment shows a close structural similarity between all four STRAP family proteins,

particularly in the predicted transmembrane domains, even though only partial sequence information was available for three of them. The STRAP-3 and STRAP-4 proteins appear to be more closely related to STRAP-2 than to STRAP-1 or each other. Specifically, STRAP-3 shows 50% identity and 69% homology to STRAP-2, versus 37% identity and 5 63% homology to STRAP-1. STRAP-4 shows 56% identity and 87% homology to STRAP-2, versus 42% identity and 65% homology to STRAP-1. STRAP-3 and STRAP-4 are 38% identical and 57% homologous to each other. These figures are estimates based upon incomplete sequence information. However, these figures suggest conservation of at least some of the transmembrane domains, suggesting common topological 10 characteristics if not functional characteristics.

**EXAMPLE 6:**

**EXPRESSION ANALYSIS OF STRAP-2 AND OTHER HUMAN STRAP FAMILY MEMBERS**

15 **Example 6A: Tissue Specific Expression of STRAP Family Members in Normal Human Tissues**  
Expression analysis of STRAP family members in normal tissues was performed by RT-PCR. All STRAP family members appeared to exhibit tissue restricted expression 20 patterns. AI139607 expression is detected in placenta and prostate after 25 cycles of amplification (FIG. 12). After 30 cycles, AI139607 expression is also detected in other tissues. R80991 expression is highest in normal liver, although expression is also detected in other tissues after 30 cycles of amplification (FIG. 13). Neither R80991, nor 25 AI139607 expression was detected in the LAPC prostate cancer xenografts by RT-PCR.

RT-PCR analysis of STRAP-2 shows expression in all the LAPC prostate cancer 30 xenografts and in normal prostate (FIG. 14, panel A). Analysis of 8 normal human tissues shows prostate-specific expression after 25 cycles of amplification (FIG. 14, panel B). Lower level expression in other tissues was detected only after 30 cycles of amplification. Northern blotting for STRAP-2 shows a pattern of 2 transcripts (approximately 3 and 8 kb 35 in size) expressed only in prostate (and at significantly lower levels in the LAPC xenografts), with no detectable expression in any of the 15 other normal human tissues analyzed (FIG. 15, panel C). Thus, STRAP-2 expression in normal human tissues appears to be highly prostate-specific.

35

**Example 6B: Expression of STRAP-2 in Various Cancer Cell Lines**

The RT-PCR results above suggested that the different STRAP family members exhibit different tissue expression patterns. Interestingly, STRAP-2, which appears very prostate-specific, seems to be expressed at lower levels in the LAPC xenografts. This is

in contrast to STRAP-1, which is highly expressed in both normal and malignant prostate tissue.

To better characterize this suggested difference in the STRAP-2 prostate cancer expression profile (relative to STRAP-1), Northern blotting was performed on RNA derived from the LAPC xenografts, as well as several prostate and other cancer cell lines, using a STRAP-2 specific probe (labeled cDNA clone 98P4B6). The results are shown in FIG. 16 and can be summarized as follows. STRAP-2 is highly expressed in normal prostate and in some of the prostate cancer xenografts and cell lines. More particularly, 5 very strong expression was observed in the LAPC-9 AD xenograft and the LNCaP cells. Significantly attenuated or no expression was observed in the other prostate cancer xenografts and cell lines. Very strong expression was also evident in the Ewing Sarcoma cell line RD-ES. Unlike STRAP-1, which is highly expressed in cancer cell lines derived 10 from bladder, colon, pancreatic and ovarian tumors, STRAP-2 showed low to non-detectable expression in these same cell lines (compare with FIG. 5). Interestingly, 15 STRAP-2 was also non-detectable in PrEC cells, which are representative of the normal basal cell compartment of the prostate. These results suggests that expression of STRAP-1 and STRAP-2 are differentially regulated. While STRAP-1 may be a gene that is generally up-regulated in cancer, STRAP-2 may be a gene that is more restricted to 20 normal prostate and prostate cancer.

**EXAMPLE 7**  
**CHROMOSOMAL LOCALIZATION OF STRAP GENES**

The chromosomal localization of STRAP-1 was determined using the GeneBridge 4 25 Human/Hamster radiation hybrid (RH) panel (Walter et al., 1994, Nat. Genetics 7:22) (Research Genetics, Huntsville AL), while STRAP-2 and the STRAP homologues were mapped using the Stanford G3 radiation hybrid panel (Stewart et al., 1997, Genome Res. 7:422).

30 The following PCR primers were used for STRAP-1:

8P1D4.1 5' ACTTTGTTGATGACCAGGATTGGA 3' (SEQ ID NO: XX)  
8P1D4.2 5' CAGAACTTCAGCACACACAGGAAC 3' (SEQ ID NO: XX)

35 The resulting STRAP-1 mapping vector for the 93 radiation hybrid panel DNAs (210000020110101000100000010111010122100011100111011010100010001000101001 021000001111001010000), and the mapping program available at the internet address <<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>>, localized the STRAP-1 gene to chromosome 7p22.3, telomeric to D7S531.

The following PCR primers were used for 98P4B6/STRAP-2:

98P4B6.1 5' GACTGAGCTGGAACTGGAAATTGT 3' (SEQ ID NO: XX)

98P4B6 2 5' TTTGAGGAGACTTCATCTCACTGG 3' (SEQ ID NO: XX)

10 The following PCR primers were used for AI139607:

AI139607.1 5' TTAGGACAACTTGATCACCAAGCA 3' (SEQ ID NO: XX)

AI138607.2 5'TGTCCAGTCCAAACTGGGTATTT3' (SEQ ID NO: XX)

15 The resulting vector (00000000100000000000000000000000100010000020000000100010000  
0001000000100010001010000010), and the mapping program available at the internet  
address <<http://www-shgc.stanford.edu/RH/rhserverformnew.html>>, maps AI139607 to  
chromosome 7q21.

The following PCR primers were used for R80991:

20 P80991-3 5' ACAAGAGGCCACCTCTGGGTGAA 3' (SEQ ID NO: XX)

BB0001\_4 5' AGTTGAGCCAGTTGCAATGGAC 3' (SEQ ID NO: XX)

In summary, the above results show that three of the putative human STRAP family members localize to chromosome 7, as is schematically depicted in FIG. 17. In particular, the STRAP-1 gene localizes to the far telomeric region of the short arm of chromosome 7, at 7p22.3, while STRAP-2 and AI139607 localize to the long arm of chromosome 7, at 7q21 (FIG. 17). R80991 maps to chromosome 2q14-q21.

35

**EXAMPLE 8:**

## IDENTIFICATION OF INTRON-EXON BOUNDARIES OF STRAP-1

Genomic clones for STRAP-1 were identified by searching GenBank for BAC clones containing STRAP-1 sequences, resulting in the identification of accession numb rs AC004969 (PAC DJ1121E10) and AC005053 (BAC RG041D11). Using the sequences

derived from the PAC and BAC clones for STRAP the intron-exon boundaries were defined (FIG. 18). A total of 4 exons and 3 introns were identified within the coding region of the STRAP gene. Knowledge of the exact exon-intron structure of the STRAP-1 gene may be used for designing primers within intronic sequences which in turn may be used  
5 for genomic amplification of exons. Such amplification permits single-stranded conformational polymorphism (SSCP) analysis to search for polymorphisms associated with cancer. Mutant or polymorphic exons may be sequenced and compared to wild type STRAP. Such analysis may be useful to identify patients who are more susceptible to aggressive prostate cancer, as well as other types of cancer, particularly colon, bladder,  
10 pancreatic, ovarian, cervical and testicular cancers.

Southern blot analysis shows that the STRAP-1 gene exists in several species including mouse (FIG. 19). Therefore, a mouse BAC library (Mouse ES 129-V release I, Genome Systems, FRAC-4431) was screened with the human cDNA for STRAP-1 (clone 10, Example 2). One positive clone, 12P11, was identified and confirmed by southern blotting (FIG. 20). The intron-exon boundary information for human STRAP may be used  
15 to identify the mouse STRAP-1 coding sequences.

The mouse STRAP-1 genomic clone may be used to study the biological role of STRAP-1  
20 during development and tumorigenesis. Specifically, the mouse genomic STRAP-1 clone may be inserted into a gene knock-out (K/O) vector for targeted disruption of the gene in mice, using methods generally known in the art. In addition, the role of STRAP in metabolic processes and epithelial cell function may be elucidated. Such K/O mice may be crossed with other prostate cancer mouse models, such as the TRAMP model  
25 (Greenberg et al., 1995, PNAS 92:3439), to determine whether STRAP influences the development and progression of more or less aggressive and metastatic prostate cancers.

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30

Throughout this application, various publications are referenced within parentheses. The disclosures of these publications are hereby incorporated by reference herein in their entireties.

35

The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any which are functionally equivalent are within the scope of the invention. Various modifications to the models and methods of the invention, in addition to those described

herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention.

**CLAIMS:**

1. An isolated STRAP-1 protein having an amino acid sequence shown in FIG. 1A (SEQ ID NO. XX).
- 5 2. An isolated polypeptide of at least 15 contiguous amino acids of the protein of claim 1.
- 10 3. An isolated polypeptide comprising an amino acid sequence which is at least 90% identical to the amino acid sequence shown in FIG. 1A (SEQ ID NO. XX) over its entire length.
- 15 4. An isolated polynucleotide selected from the group consisting of (a) a polynucleotide having the sequence as shown in FIG. 1A (SEQ ID NO. XX), wherein T can also be U; (b) a polynucleotide encoding a STRAP-1 polypeptide whose sequence is encoded by the cDNA contained in plasmid 8P1D4 clone 10.1 as deposited with American Type Culture Collection as Accession No. 98849; and (c) a polynucleotide encoding the STRAP-1 protein of claim 1.
- 20 5. An isolated polynucleotide which is fully complementary to a polynucleotide according to claim 4.
6. A recombinant expression vector which contains a polynucleotide according to claim 4.
- 25 7. A host cell which contains an expression vector according to claim 6.
8. A process for producing a STRAP-1 protein comprising culturing a host cell of claim 7 under conditions sufficient for the production of the polypeptide and recovering the STRAP-1 protein from the culture.
- 30 9. A STRAP-1 polypeptide produced by the method of claim 8.
10. An isolated STRAP-2 protein comprising the amino acid sequence shown in FIG. 9 (SEQ ID NO. XX).
- 35 11. An isolated polypeptide of at least 15 contiguous amino acids of the protein of claim 10.

12. An isolated polynucleotide selected from the group consisting of (a) a polynucleotide having the sequence as shown in FIG. 9 (SEQ ID NO. XX), wherein T can also be U; and (b) polynucleotide encoding the STRAP-2 protein of claim 10.
- 5 13. An isolated polynucleotide which is fully complementary to a polynucleotide according to claim 12.
- 10 14. An antibody which (a) immunohistochemically stains 293T cells transfected with an expression plasmid encoding STRAP-1 according to claim 1, wherein the transfected 293T cells express STRAP-1 protein; and, (b) does not immunohistochemically stain untransfected 293T cells.
- 15 15. The antibody of claim 14, wherein the 293T cells are transfected with an expression plasmid containing the STRAP-1 coding sequence within plasmid 8P1D4 clone 10.1 as deposited with American Type Culture Collection as Accession No. 98849.
16. An antibody which immunospecifically binds to the STRAP-1 protein of claim 1 or the polypeptide of claim 2.
- 20 17. A monoclonal antibody according to claim 16.
18. A fragment of the antibody of claim 17.
19. A recombinant protein comprising the antigen binding domain of the antibody of claim 17.
- 25 20. The antibody of claim 17 which is labeled with a detectable marker.
21. The monoclonal antibody of claim 17 which is conjugated to a toxin.
- 30 22. The monoclonal antibody of claim 17 which is conjugated to a therapeutic agent.
23. The antibody fragment of claim 18 which is labeled with a detectable marker.
- 35 24. The recombinant protein of claim 19 which is labeled with a detectable marker.
25. An antibody which immunospecifically binds to the STRAP-2 protein of claim 10 or the polypeptide of claim 11.

26. A monoclonal antibody according to claim 25.
27. The antibody of claim 26 which is labeled with a detectable marker.
- 5 28. The monoclonal antibody of claim 26 which is conjugated to a toxin.
29. The monoclonal antibody of claim 26 which is conjugated to a therapeutic agent.
- 10 30. An assay for detecting the presence of a STRAP-1 protein in a biological sample comprising contacting the sample with an antibody of claim 20, an antibody fragment of claim 23, or a recombinant protein of claim 24, and detecting the binding of STRAP-1 protein in the sample thereto.
- 15 31. An assay for detecting the presence of a STRAP-2 protein in a biological sample comprising contacting the sample with an antibody of claim 27, and detecting the binding of STRAP-2 protein in the sample thereto.
32. An assay for detecting the presence of a STRAP-1 polynucleotide in a biological sample, comprising
- 20 (a) contacting the sample with a polynucleotide probe which specifically hybridizes to the STRAP-1 cDNA contained within plasmid 8P1D4 clone 10.1 as deposited with American Type Culture Collection as Accession No. 98849, or the polynucleotide as shown in FIG. 1A (SEQ ID NO. XX), or the complements thereof; and
- 25 (b) detecting the presence of a hybridization complex formed by the hybridization of the probe with STRAP-1 polynucleotide in the sample, wherein the presence of the hybridization complex indicates the presence of STRAP-1 polynucleotide within the sample.
- 30 33. An assay for detecting the presence of a STRAP-2 polynucleotide in a biological sample, comprising
- 35 (a) contacting the sample with a polynucleotide probe which specifically hybridizes to a polynucleotide of claim 12 or its complement; and
- (b) detecting the presence of a hybridization complex formed by the hybridization of the probe with STRAP-2 polynucleotide in the sample, wherein the presence of the

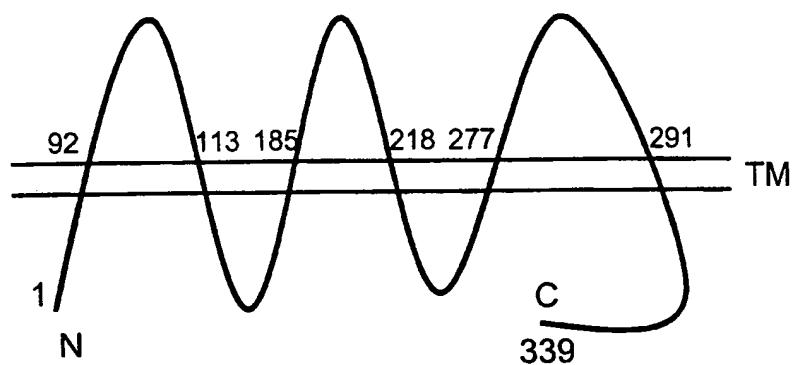
hybridization complex indicates the presence of STRAP-2 polynucleotide within the sample.

34. An assay for detecting the presence of STRAP-1 mRNA in a biological sample  
5 comprising:
  - (a) producing cDNA from the sample by reverse transcription using at least one primer;
  - 10 (b) amplifying the cDNA so produced using STRAP-1 polynucleotides as sense and antisense primers to amplify STRAP-1 cDNAs therein;
  - (c) detecting the presence of the amplified STRAP-1 cDNA,  
15 wherein the STRAP-1 polynucleotides used as the sense and antisense probes are capable of amplifying the polynucleotide shown in FIG. 1A (SEQ ID NO. XX).
35. An assay for detecting the presence of STRAP-2 mRNA in a biological sample  
comprising:  
20 (a) producing cDNA from the sample by reverse transcription using at least one primer;  
  
(b) amplifying the cDNA so produced using STRAP-2 polynucleotides as sense and  
25 antisense primers to amplify STRAP-2 cDNAs therein;  
  
(c) detecting the presence of the amplified STRAP-2 cDNA,  
wherein the STRAP-2 polynucleotides used as the sense and antisense probes are  
30 capable of amplifying the polynucleotide shown in FIG. 9 (SEQ ID NO. XX).
36. A composition for the treatment of prostate cancer comprising an antibody  
according to claim 17, 21 or 22, wherein the antibody binds to an extracellular  
domain of STRAP-1.  
35

37. A composition for the treatment of colon cancer comprising an antibody according to claim 17, 21 or 22, wherein the antibody binds to an extracellular domain of STRAP-1.
- 5 38. A composition for the treatment of bladder cancer comprising an antibody according to claim 17, 21 or 22, wherein the antibody binds to an extracellular domain of STRAP-1.
- 10 39. A composition for the treatment of prostate cancer comprising an antibody according to claim 26 or 28, wherein the antibody binds to an extracellular domain of STRAP-2.

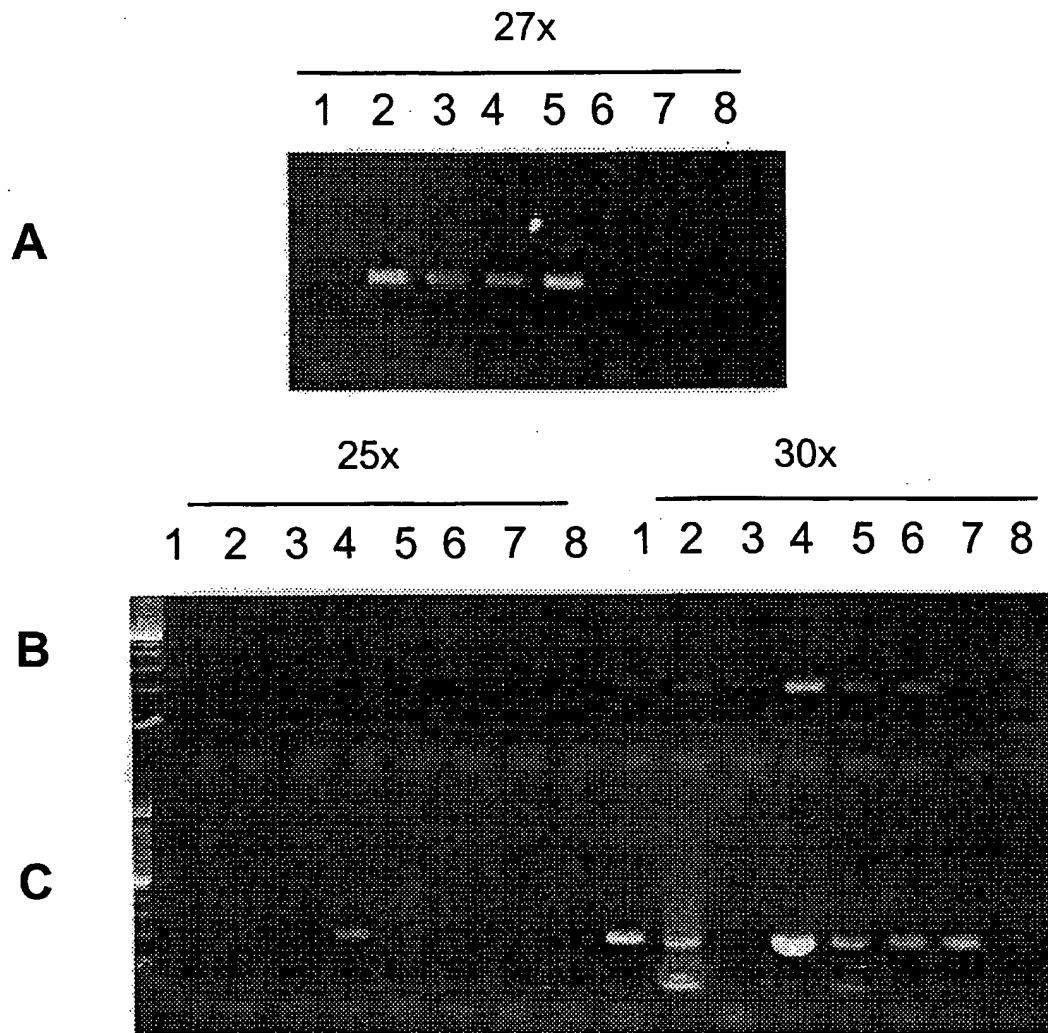
**FIG. 1B**

Extracellular



# FIG. 1C

5' GGC GGA GGC GGA GGC GGG CGA GGG GCG GGG AGC GCC GCC TGG AGC GCG  
GCA GGT CAT ATT GAA CAT TCC AGA TAC CTA TCA TTA CTC GAT GCT GTT GAT  
AAC AGC AAG 3'

**FIG. 2****Panels:****A**

1. Brain
2. Prostate
3. LAPC-4 AD
4. LAPC-4 AI
5. LAPC-9 AD
6. HeLa
7. Murine cDNA
8. Neg. control

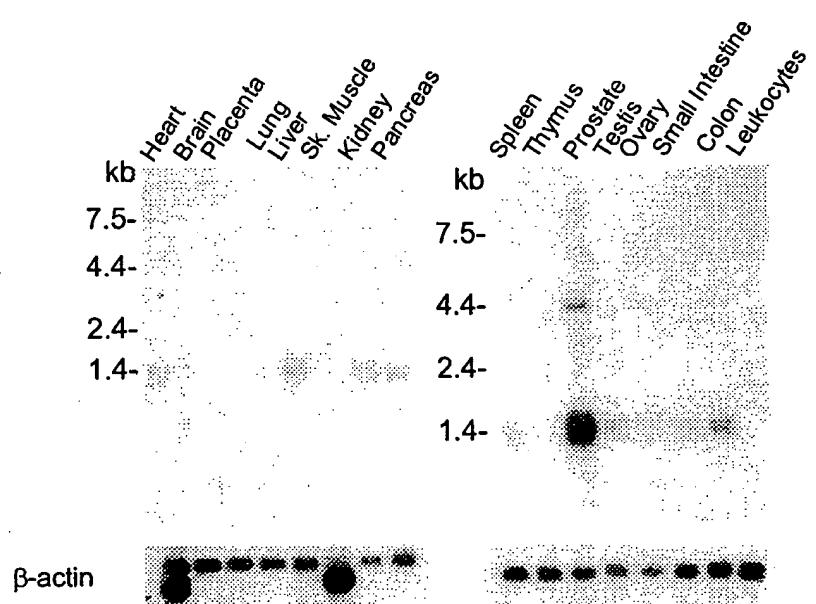
**B**

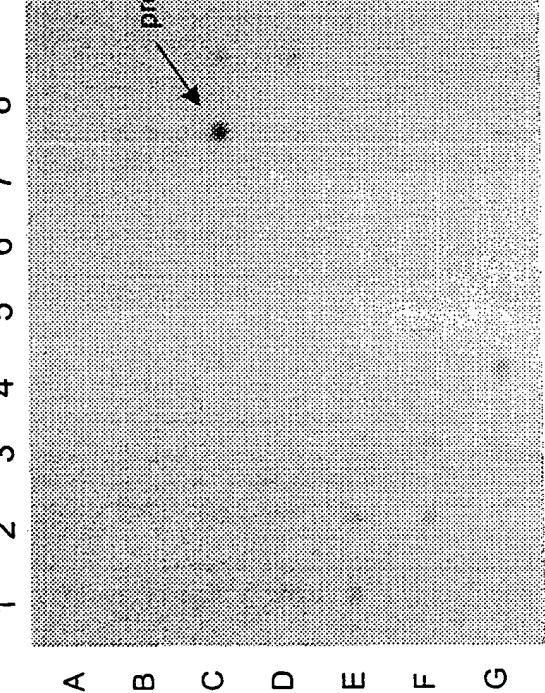
1. Brain
2. Heart
3. Kidney
4. Liver
5. Lung
6. Pancreas
7. Placenta
8. Skeletal Muscle

**C**

1. Colon
2. Ovary
3. Leukocytes
4. Prostate
5. Small Intestine
6. Spleen
7. Testis
8. Thymus

## FIG. 3A



**FIG. 3B**

A1 brain	D1 testis
A2 amygdala	D2 ovary
A3 caudate nucleus	D3 pancreas
A4 cerebellum	D4 pituitary gland
A5 cerebral cortex	D5 adrenal gland
A6 frontal lobe	D6 thyroid gland
A7 hippocampus	D7 salivary gland
A8 medulla oblongata	D8 mammary gland
B1 occipital lobe	E1 kidney
B2 putamen	E2 liver
B3 substantia nigra	E3 small intestine
B4 temporal lobe	E4 spleen
B5 thalamus	E5 thymus
B6 sub-thalamic nucleus	E6 peripheral leukocytes
B7 spinal cord	E7 lymph node
	E8 bone marrow
C1 heart	F1 appendix
C2 aorta	F2 lung
C3 skeletal muscle	F3 trachea
C4 colon	F4 placenta
C5 bladder	
C6 uterus	
<b>C7 prostate</b>	G1 fetal brain
C8 stomach	G2 fetal heart
	G3 fetal kidney
	<b>G4 fetal liver</b>
	G5 fetal spleen
	G6 fetal thymus
	G7 fetal lung

**FIG. 4**

GGGGCCCGCACCTCTGGCAGCAGCGCAGCCGAGACTCACGGTCAAGCTAAGGCGAAGAGTGGGTGGCTGAAGCC  
ATACTATTATAGAATTAATGAAAGCAGAAAAGACATCACAAACCAAGAAGAACTTTGGAAAATGARGCCTAGG  
AGAAATTAGAAGAAGACGATTATTCATAAGGACACGGGAGAGACCAGCATGCTAAAAGACCTGTGCTTGC  
ATTCACCAACAGCCATGCTGATGAATTGACTGCCCTCAGAACTTCAGCACACAGGAACCTTTCCACA  
GTGGCAGTGCCTAAATAGCTGCTATTATAGCATCTCTGACTTTCTTACACTCTCTGAGGGAAAGTAATT  
CACCCCTAGCAACTTCCATCAACAAATTAAATTCAATCCTGGTCACTCAACAAAGTCTTCCAATGG  
TTTCCATCACTCTGGCATTGGTTACCTGCCAGGTGATAGCAGCAATTGTCACACTCATAATGAAACAA  
GTATAAGAAGTTCCACATTGGTGGATAAGTGGATGTTAACAGAAAGCAGTTGGCTTCAGTTTTTT  
GCTGTACTGCATGCAATTATAGCTGTCTTACCCATGAGGGCATCCTACAGATAACAGTTGCTAAACTGGCATT  
ATCAACAGGTCCAACAAAATAAGAAGATGCCTGGATTGAGCATGATGTTGGAGAATGGAGATTATGTC  
GGGAATTGTTGGGATTGCAACTTGCTCTGGCTGTGACATCTATTCCATCTGTGAGTGA  
CTTTGACATGGAGAATTTCACATATTCAAGTAAATAATATAAAACCTAACAGGTAATCTTCTTTGTGTTATGAT  
ATAGAATATGTTGACTTTACCCATAAAAAAAACAAATGTTTCAACAGCAAGATCTTACACTTGTCCAATT  
ATAATGTCCTCCTGTTGTTCCCTATTGCTCTAATTAGGACAAGTGTCTCTAGACATAAAAGGCAT  
TAAAATATTCTTGTGTTTTTTGTTGTTGTTGTTGTTGTTGAGATGAAGTCTCG  
CTCTGTTGCCATGCTGGAGTACAGTGGCACGATCTGGCTACTGCAACCTGCCCTGGTTGAGCGATT  
TCTTGCCCTAGCCTGAGTAGCTGGATTACAGGCACCCATCACCATGTCAGCTAATTGTTGTTAGTA  
GAGACAGGGTTTCCATGTTGCCAGGCTGGCTCGATCTCTGACCTCAATGATCCGCCACCTGCCCTCC  
AAAGTGCTGGGATGACAGTTGTGAGCCACCACACTCAGCCTGCTTTCTAATATTGAAACTGTTAGACAATT  
GCTACCCATCTAATGTAATTAGGAATCCATGATGTTATTATTCTTAAAGGGAAATTTTAC  
TGTCACCTGAATTAGTAATGCCATTGTTACACAATTGCACTTCCAGAAACAAA  
ACTTACTCACTGAAGTTCTAAATATTCTGTAATTAAA  
ACTATCTCAGGTTATCTGCTGAGTGTGCCAGAATCACTCTGGATCCTGCTGACAAGATTCAAAGGACTAA  
TTAATTCACTGAAACACTGCCATTACCGTTATGGTAGACATCTTGGAAATTCCACAAGGTCA  
CGCAACTATCCCTCTACATGTCACACGTATACTCCAACACTTATTAGGCATCTGATTAGTTGGAAAGTATGC  
CTCCATCTGAATTAGTCCAGTGTGGCTTAGAGTTGGTACAACATTCTCACAGAATTCTTAATTGTTAGGTT  
CCTGATAACCACTGGAGTTCTTGGCCTCATTAAATAGCTTCCACACATTGCTCTGCCTGTTACACATATGA  
TGAACACTGCTTTAGACTCATTAGGAATTAGGACTGCATCTGACAACGTGAGCCTATTCTACTATGTACA

ATACCTAGCCATAATAGGTATAACAATACACATTTGGTAAAACATAATTCAACCAATGACATGTATTTCAACT  
AGTAACCTAGAAATGTTCACTTAAATCTGAGAACTGGTACACTACAAGTTACCTTGGAGATTCAATATGAAA  
ACGCAAACCTAGCTATTGATTGTATTCACTGGACTTAAGAATGCGCCTGAATAATTGTGAGTTCGATTTGTTCT  
GGCAGGCTAATGACCATTCCAGTAAAGTGAATAGAGGTAGAAGTCGTATAAAAGAGGTGTTGTCAGAACACCGT  
TGAGATTACATAGGTGAACAACATTTTAAGCAACTTATTGTGAGTCAGAAAGCATCCAATGCAGGCTGAA  
ATGTTCATCACATCTCTGGATCTCTATTGTGAGACATTGAAAAAATTGTTCATATTTCATGTTATC  
AGAATATTGATTTTAAAAACATAGGCCAAGTCAATTCACTTATTCAATTCAAAATCAGAGTGAATCA  
CATTAGTCGCCCTCACAACTGATAAAGATCACTGAAGTCAAATTGATTTGCTATAATCTCAATCTACCTAT  
TTAATTGAGAATCTAAAATGTACAATCATTGTGTTGATTCTGCAGTGATCCTGCTATAAGTAAGACTCAGTCCT  
GATTTAGGTATCTGTGAAAAGCAGAATTAAGACAATACACAAGAGACAAGCACAAAAATAATCATAAG  
GGGATGAACAAAATGGTGGAGAAAGAGTAGACAAGTTTGATCACCTGCCCTCAAAGAAAGGCTGTGAATTTG  
TTCACCTAGACAGCTGGAGACAAGAAATTACCCAAAGTAAGGTGAGGAGTAGGCAAAAGAGCAGAAAGATG  
TGAATGGACATTGTTGAGAAATGTGATAGGAAACATAGATAAAGGATTCCAAGCAACAGAGCATATCCAG  
ATGAGGTAGGATGGGATAAAACTCTTATTGAACCAATCTCACCAATTGTTTCTTGTGAGCAAGCTAGGA  
ATTGTTCCCTCTACTGGCACAATACACGCATTGATTTGCCCTGGAATAAGTGGATAGATAAAACAAATTG  
TATGGTATACACCTCCAACCTTATGATAGCTGTTTCCCTCAATTGTTGCTGTATTTAAAGCATACTATT  
CCTGCCATGCTTGAGGAAGAGATACTGAAGATTAGACATGGTGGGAAGACGTACCAAAATTACAAACTGAG  
ATATGTTCCAGTTGTAAGAATTACTGTTACACACATTGTTCAATATTGATATTTTATCACCACATTCA  
AGTTGTATTGTTAATAAAATGATTATTCAAGGAAAAA

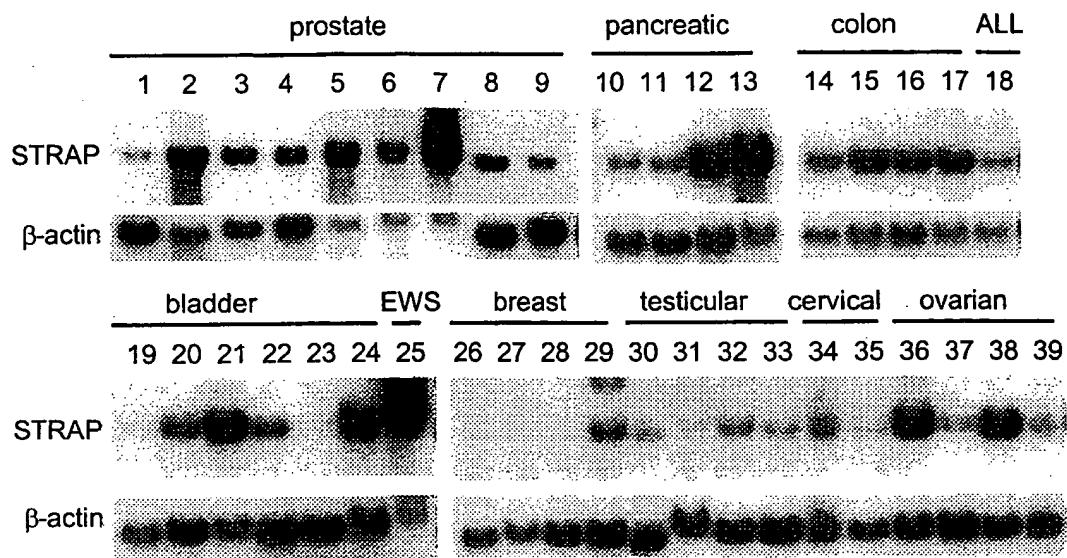
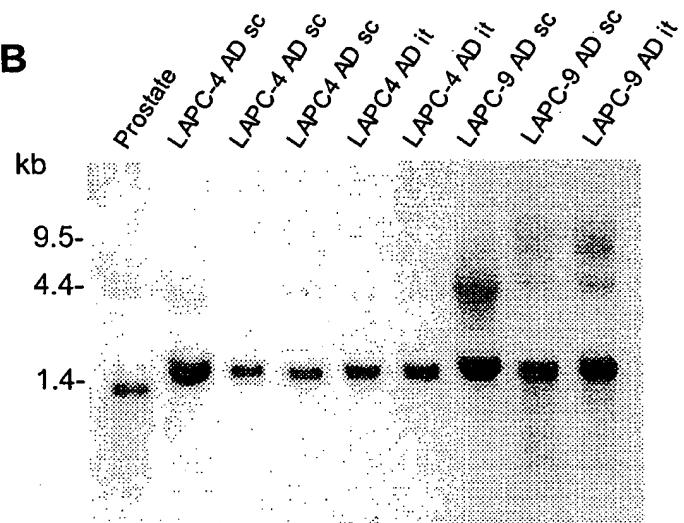
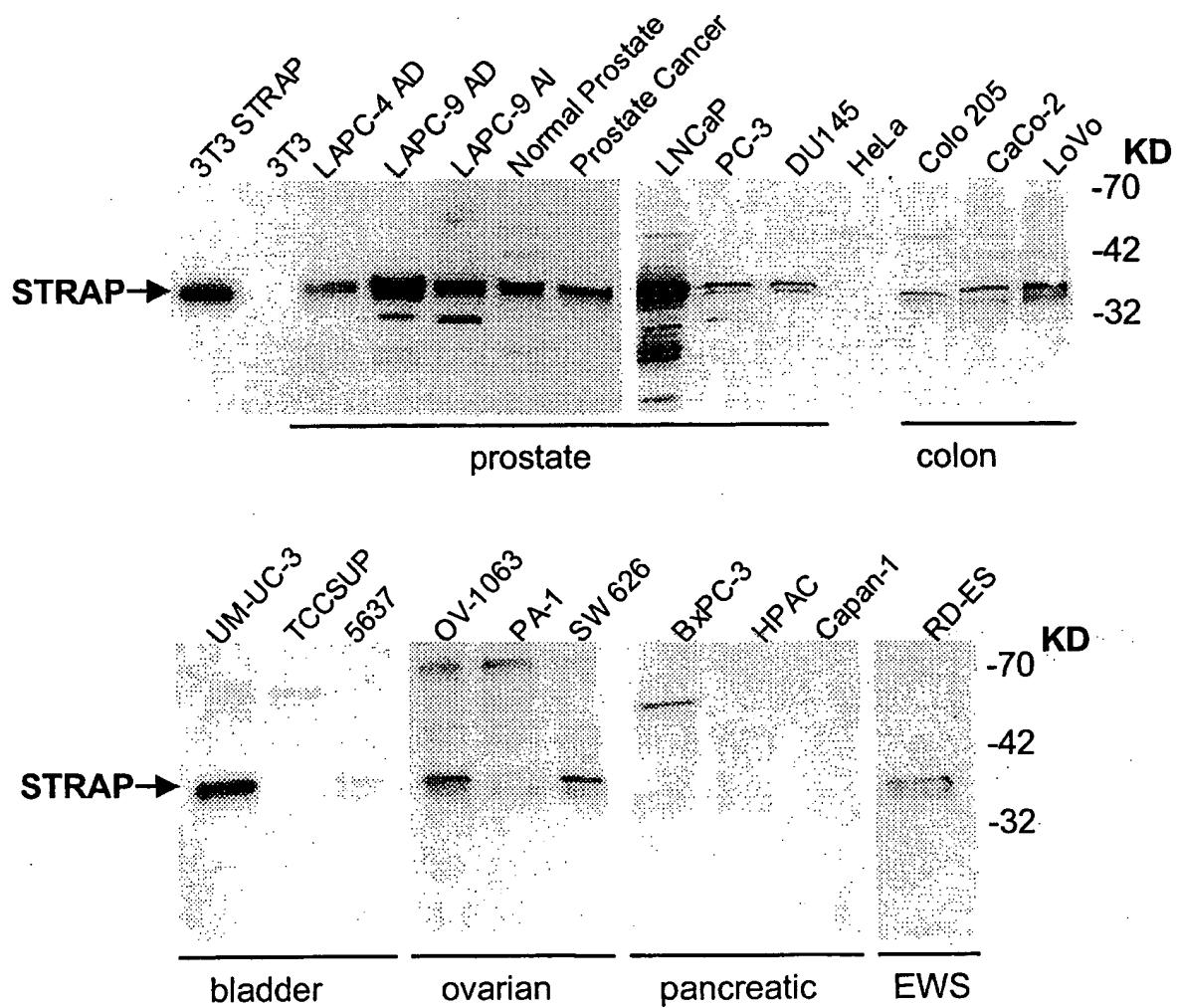
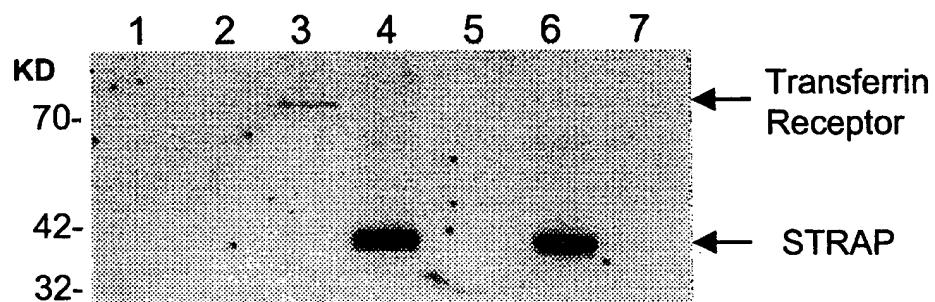
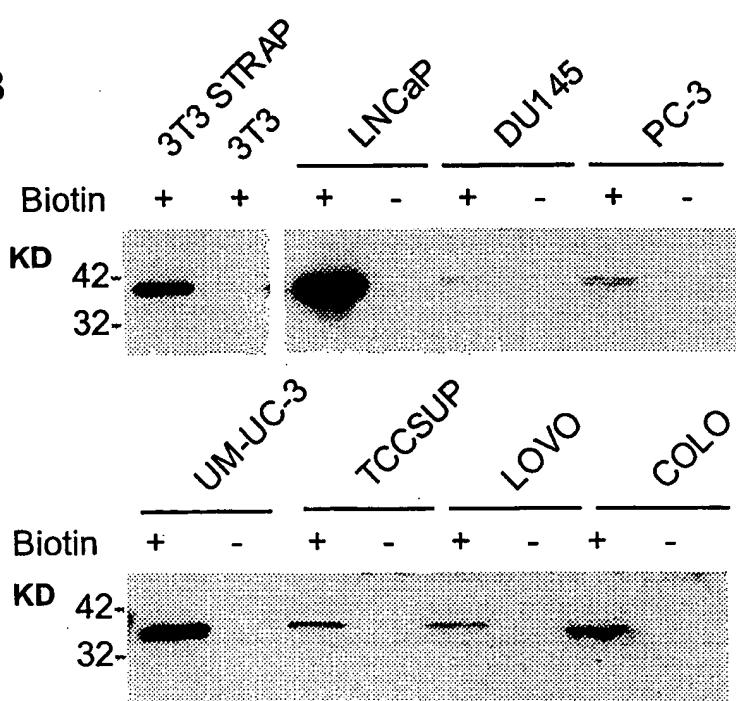
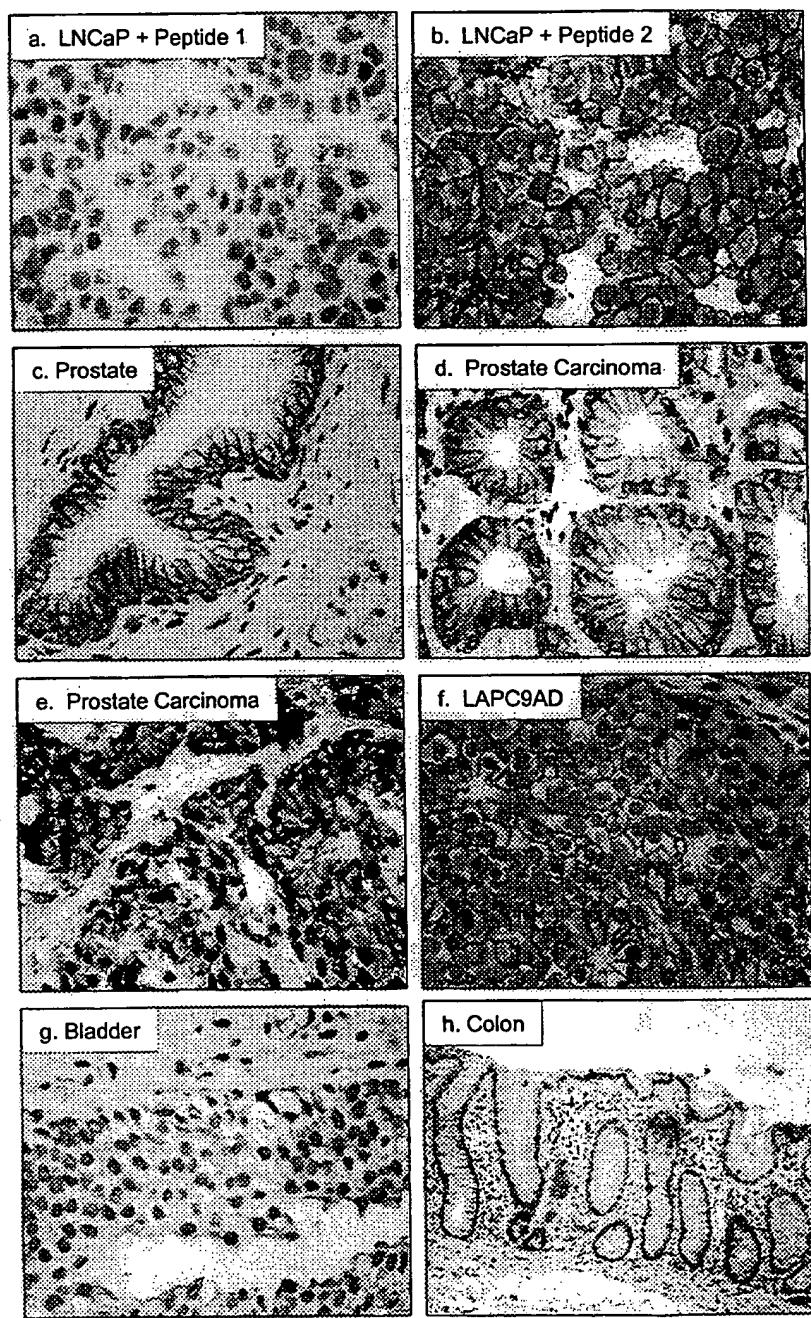
**FIG. 5****A****B**

FIG. 6



**FIG. 7****A****B**

**FIG. 8**

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## FIG. 9

	10	19	28	37	46	55												
5'	GAC	TTT	TAC	AAA	ATT	CCT	ATA	GAG	ATT	GTG	AAT	AAA	ACC	TTA	CCT	ATA	GTT	GCC
	Asp	Phe	Tyr	Lys	Ile	Pro	Ile	Glu	Ile	Val	Asn	Lys	Thr	Leu	Pro	Ile	Val	Ala
	64	73	82	91	100	109												
	ATT	ACT	TTG	CTC	TCC	CTA	GTA	TAC	CTC	GCA	GGT	CTT	CTG	GCA	GCT	GCT	TAT	CAA
	Ile	Thr	Leu	Leu	Ser	Leu	Val	Tyr	Leu	Ala	Gly	Leu	Leu	Ala	Ala	Tyr	Gln	
	118	127	136	145	154	163												
	CTT	TAT	TAC	GGC	ACC	AAG	TAT	AGG	AGA	TTT	CCA	CCT	TGG	TTG	GAA	ACC	TGG	TTA
	Leu	Tyr	Tyr	Gly	Thr	Lys	Tyr	Arg	Arg	Phe	Pro	Pro	Trp	Leu	Glu	Thr	Trp	Leu
	172	181	190	199	208	217												
	CAG	TGT	AGA	AAA	CAG	CTT	GGA	TTA	CTA	AGT	TTT	TTC	TTC	GCT	ATG	GTC	CAT	GTT
	Gln	Cys	Arg	Lys	Gln	Leu	Gly	Leu	Leu	Ser	Phe	Phe	Phe	Ala	Met	Val	His	Val
	226	235	244	253	262	271												
	GCC	TAC	AGC	CTC	TGC	TTA	CCG	ATG	AGA	AGG	TCA	GAG	AGA	TAT	TTG	TTT	CTC	AAC
	Ala	Tyr	Ser	Leu	Cys	Leu	Pro	Met	Arg	Arg	Ser	Glu	Arg	Tyr	Leu	Phe	Leu	Asn
	280	289	298	307	316	325												
	ATG	GCT	TAT	CAG	CAG	GTT	CAT	GCA	AAT	ATT	GAA	AAC	TCT	TGG	AAT	GAG	GAA	GAA
	Met	Ala	Tyr	Gln	Gln	Val	His	Ala	Asn	Ile	Glu	Asn	Ser	Trp	Asn	Glu	Glu	Glu
	334	343	352	361	370	379												
	GTT	TGG	AGA	ATT	GAA	ATG	TAT	ATC	TCC	TTT	GGC	ATA	ATG	AGC	CTT	GGC	TTA	CTT
	Val	Trp	Arg	Ile	Glu	Met	Tyr	Ile	Ser	Phe	Gly	Ile	Met	Ser	Leu	Gly	Leu	Leu
	388	397	406	415	424	433												
	TCC	CTC	CTG	GCA	GTC	ACT	TCT	ATC	CCT	TCA	GTG	AGC	AAT	GCT	TTA	AAC	TGG	AGA
	Ser	Leu	Leu	Ala	Val	Thr	Ser	Ile	Pro	Ser	Val	Ser	Asn	Ala	Leu	Asn	Trp	Arg
	442	451	460	469	478	487												
	GAA	TTC	AGT	TTT	ATT	CAG	TCT	ACA	CTT	GGA	TAT	GTC	GCT	CTG	CTC	ATA	AGT	ACT
	Glu	Phe	Ser	Phe	Ile	Gln	Ser	Thr	Leu	Gly	Tyr	Val	Ala	Leu	Leu	Ile	Ser	Thr
	496	505	514															
	TTC	CAT	GTT	TTA	ATT	TAT	GGA	TGG	AAA	CGA	GCT	3'						
	Phe	His	Val	Leu	Ile	Tyr	Gly	Trp	Lys	Arg	Ala							

**FIG. 10**

STRAP-2, AA508880 (NCI\_CGAP Pr6)  
ggtcgactttccttattccttgttagagatctgattcatccatatgctagaaaaccaacagagtgacttttaca  
aaatccctatagatgtgaaaaaaacccatccatatagttgcattatcttgcctccctagtatataccttgcagg  
tcttcggcagctgttataactttattacggcacaatgtataggagattttcaccccttggaaacttgcgttgcagg  
cagtgttagaaaaacagcttgcattactaattgtttttcgctatgtccatgtgcctacagcctgtcttaccgat  
tgagaaaggctcagagagat

STRAP-2, 98P4B6 SSH fragment  
TTTGCGAGCTTGAGATACCCAGACTGAGCTGGAACTGGAATTGTCTTCCTATTGACTCTACTTCTTTAAAGCG  
GCTGCCCATTCATTCTCAGGTGCTTGCAGTTAGGTGTACATGTGACTGAGTGTGGCAGTGAGATGAAGTC  
TCTCTAAAGGAAGGGCAGCATGTGTCTTTT

AI139607 (testis EST)  
aagaaggagaatccatttagcacccctcagcctggctcagtgattcatatgtggcttggaaatacttgggttt  
ttctgtttgactcttggaaatcactcttgcacatctgttagacaatgcagtcactaaatggagagatccgatttg  
ccagtc当地有三个不同的序列，我将它们全部显示出来，以确保完整性。  
agcccttc当地有三个不同的序列，我将它们全部显示出来，以确保完整性。  
tcaagtttgc当地有三个不同的序列，我将它们全部显示出来，以确保完整性。  
acactaaaaaaa当地有三个不同的序列，我将它们全部显示出来，以确保完整性。

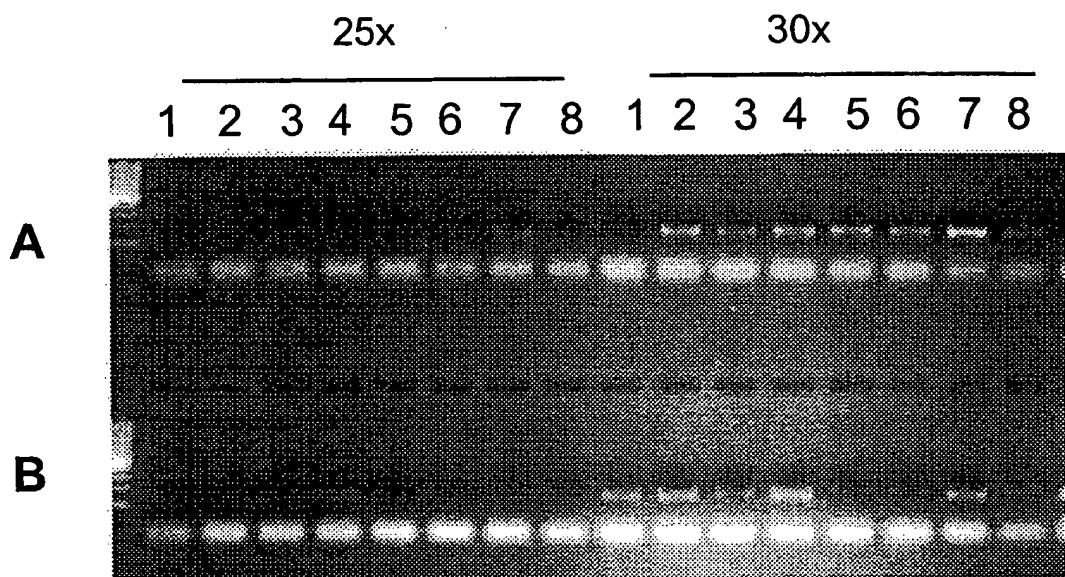
R80991 (placental EST)  
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tgacacctgctggcgatggagatctaaccttccctgggagtgtggccctcggcacgttgcctgtggccg  
ggccntctgtggacacactncacacgctcacctacggctggaccgcgccttcgaggagagccgtacaaggtc  
tacccntccccaccttcacgntcacgctgtggccctgcgttcatcctggccaaagccctgtttntac  
tgccttgattcagccnaga

## FIG. 11A

STRAP-1	106	FYKIPILVINKVLPMSITLLALVYLPGVIAAIVQLHNGTKYKKFPHWLDKWMILTRKQFG
STRAP-2	2	FYKIPIEIVNKTLPIVAITLLSLVYLAGLAAAYQLYYGTKYRRFPPWLETWLQCRKQLG
	*****	*****
STRAP-1	166	LLSFFFAVLHAIYSLSYPMRRSYRYKLLNWAYQQVQQNKEADAWIEHDVWRMEIYVSLGIV
STRAP-2	62	LLSFFFAMVHVAYSCLPMRRSERYLFLNMAYQQVHANIENSWNEEEVWRIEMYISFGIM
	*****	*****
STRAP-1	226	GLAILALLAVTSIPSVDSDLTWREFHYIQSKLGIVSLLLGTIHALIFAWNK
STRAP-2	122	SLGLLSSLLAVTSIPSVDNALNWREFSFIQSTLGYVALLISTFHVLITYGWKR
	*****	*****

## FIG. 11B

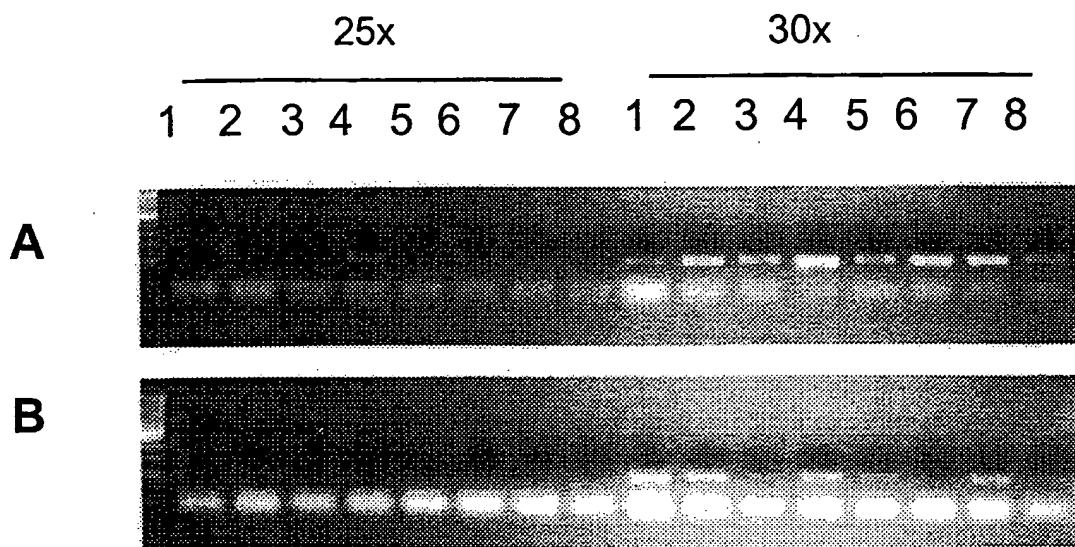
1	15	16	30	31	45	46	60	61	75	76	90
STRAP-1	MESRKDITNQEEELWK	MKPRRNLEDDYLHK	DTGETSMLRPVLLH	LHQTAHADEPFCOPSE	LQHQELFPOMHLP	KIAAIIASLSTFLYL					
STRAP-2	-	-	-	-	-	-	-	-	-	-	0
STRAP-3	-	-	-	-	-	-	-	-	-	-	0
STRAP-4	-	-	-	-	-	-	-	-	-	-	0
91	105	106	120	121	135	136	150	151	165	166	180
STRAP-1	LREVTHPLATSHQQY	[FYKIPPI]VINKVLPW	VSTTLLAVVYV[PPV]	[PPIVQDHN]GNTKYKK							
STRAP-2	-	-	-	-	-	-	-	-	-	-	76
STRAP-3	-	-	-	-	-	-	-	-	-	-	0
STRAP-4	-	-	-	-	-	-	-	-	-	-	0
181	195	196	210	211	225	226	240	241	255	256	270
STRAP-1	SYPPMRSYKRTKLLW	AYQQVQONKEDAWIE	HDV[ARMETIVS]GTY	QIAIA[LLAVT]SPLS	VPSDLT[WR]EPFVQS	KIGIVSLLGIGRIR					
STRAP-2	CLPPMRSYKRTKLLW	AYQQVHANIENSWE	EEV[Q]TETTSFQTM	SIGL[SLAVT]SPLS	VSNALNWREFSFVQS	TGIVALLISTPFTV					
STRAP-3	-	-	-	-	-	-	-	-	-	-	68
STRAP-4	-	-	-	-	-	-	-	-	-	-	82
271	285	286	300	301	315	316	330	331	345	346	360
STRAP-1	IPAWNKWIDIKQFW	Y[PPPT]MIAVFLPIV	[V]L[PPK]SILFLPCQRK	KILKIRHGWEDVTKI	NKTEICSQL	339					
STRAP-2	IYGWKRA	-	-	-	-	-	-	-	173		
STRAP-3	VYGGKRFSPSNLRW	YLPAAVYGLIIPCT	[V]L[PPK]VLTIMPCTP	TLT[TR]PQGNERNSKH	-	-	128				
STRAP-4	TYGMTRAFEEESYKF	Y[PPPT]FTXILLVPCV	RS[SMKAKF]XLPCIQ	P	-	-	128				

**FIG. 12****A**

1. Brain
2. Heart
3. Kidney
4. Liver
5. Lung
6. Pancreas
7. Placenta
8. Skeletal Muscle

**B**

1. Colon
2. Ovary
3. Leukocytes
4. Prostate
5. Small Intestine
6. Spleen
7. Testis
8. Thymus

**FIG. 13****A**

1. Brain
2. Heart
3. Kidney
4. Liver
5. Lung
6. Pancreas
7. Placenta
8. Skeletal Muscle

**B**

1. Colon
2. Ovary
3. Leukocytes
4. Prostate
5. Small Intestine
6. Spleen
7. Testis
8. Thymus

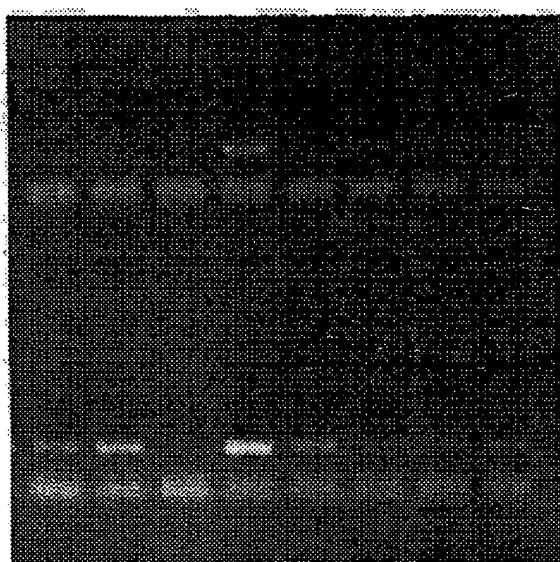
**FIG.14**

26x

**A****B**

1 2 3 4 5 6 7 8

25x



30x

**A**

1. Brain
2. Prostate
3. LAPC-4 AD
4. LAPC-4 AI
5. LAPC-9 AD
6. HeLa
7. Murine cDNA
8. Neg. control

**B**

1. Colon
2. Ovary
3. Leukocytes
4. Prostate
5. Small Intestine
6. Spleen
7. Testis
8. Thymus

FIG. 15

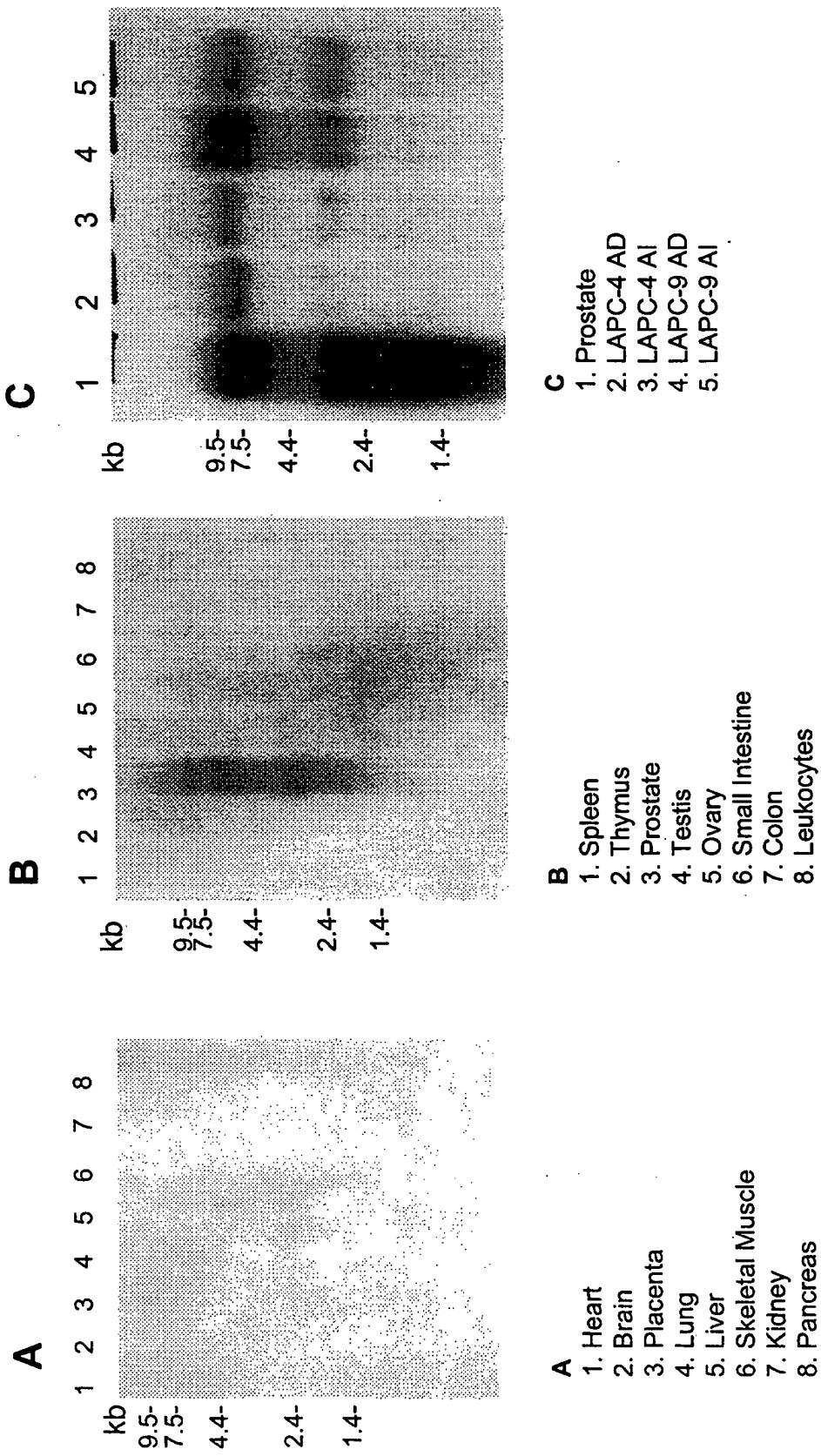
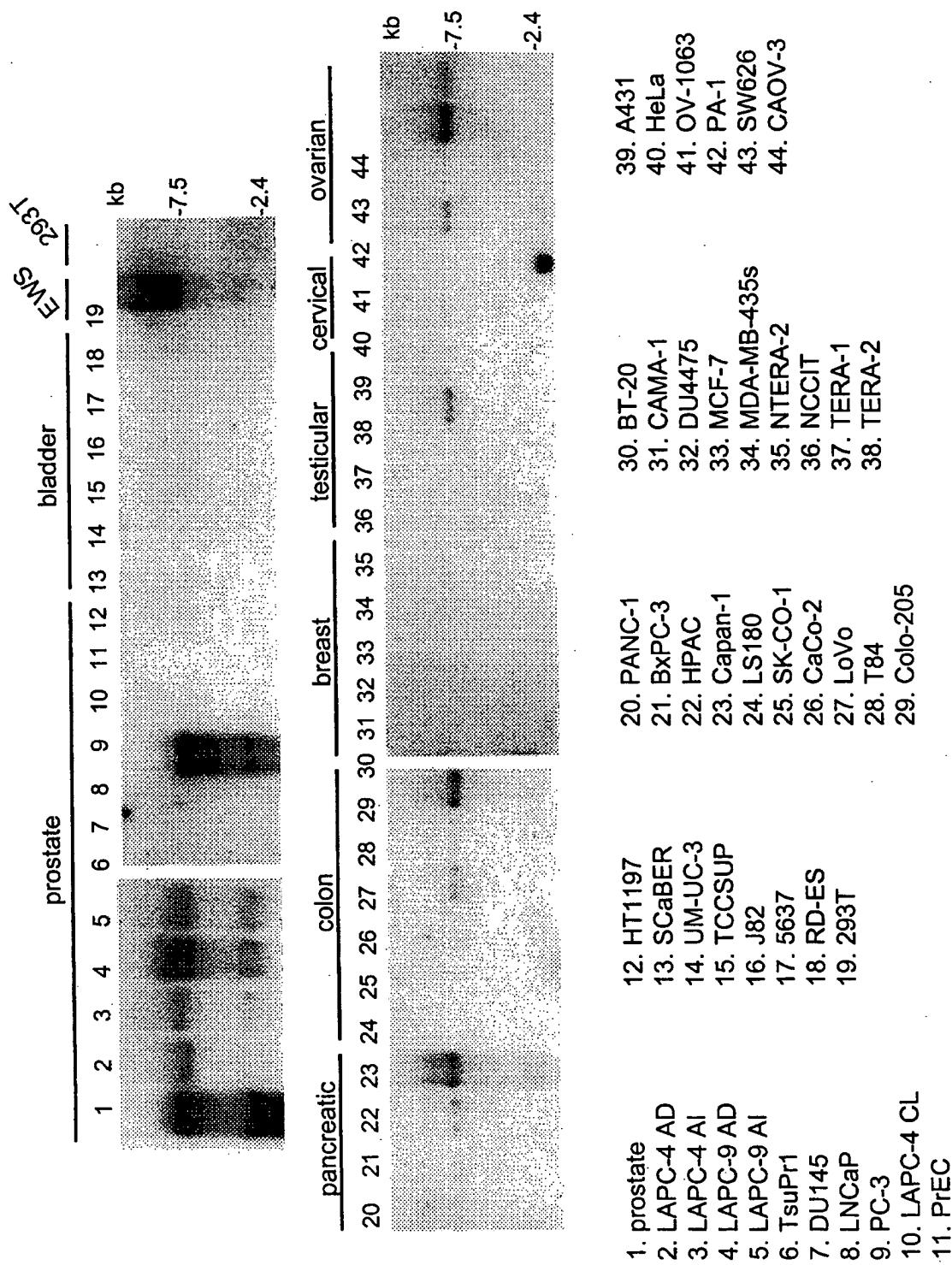


FIG. 16



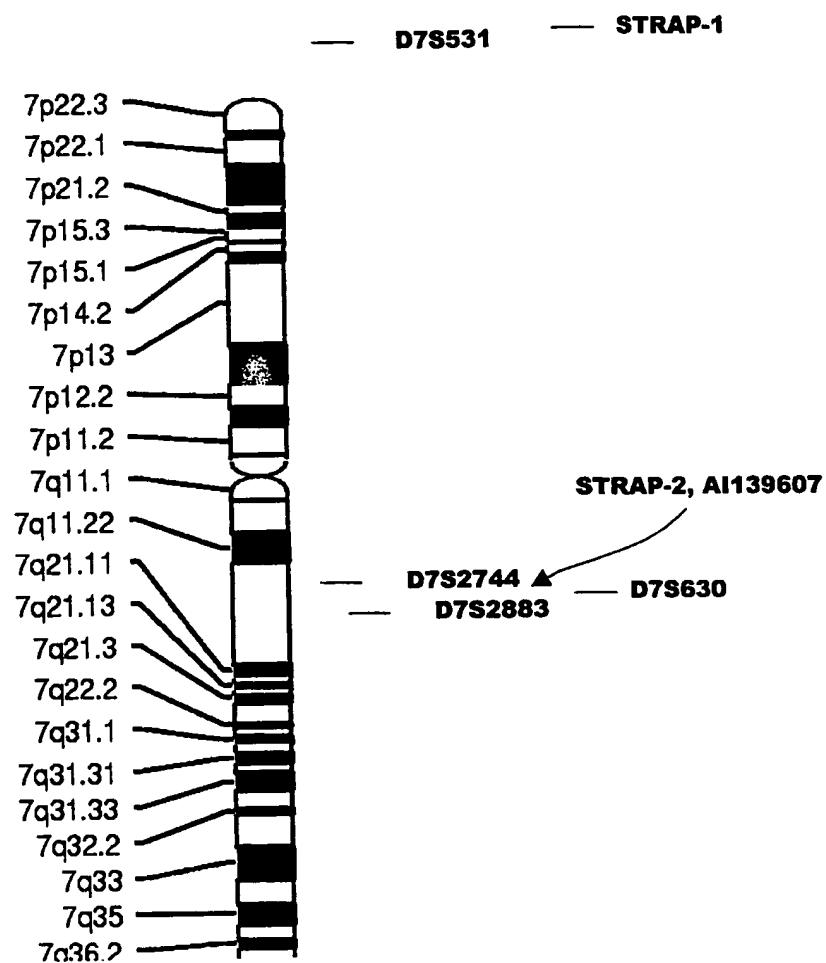
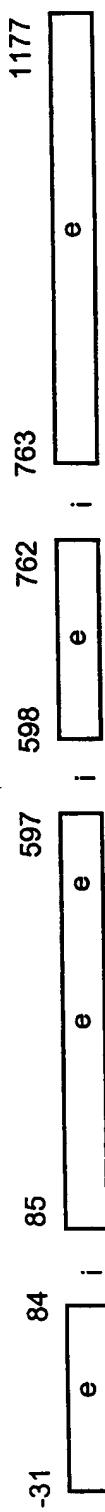
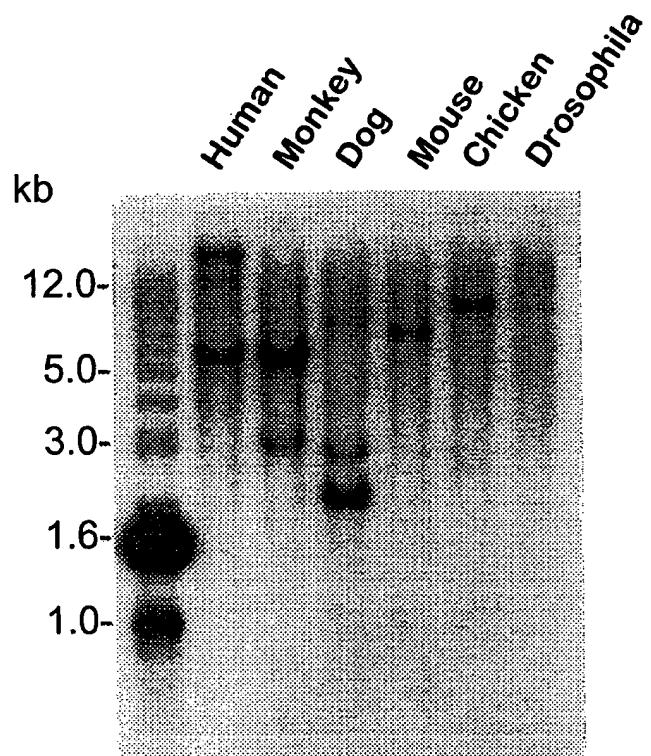
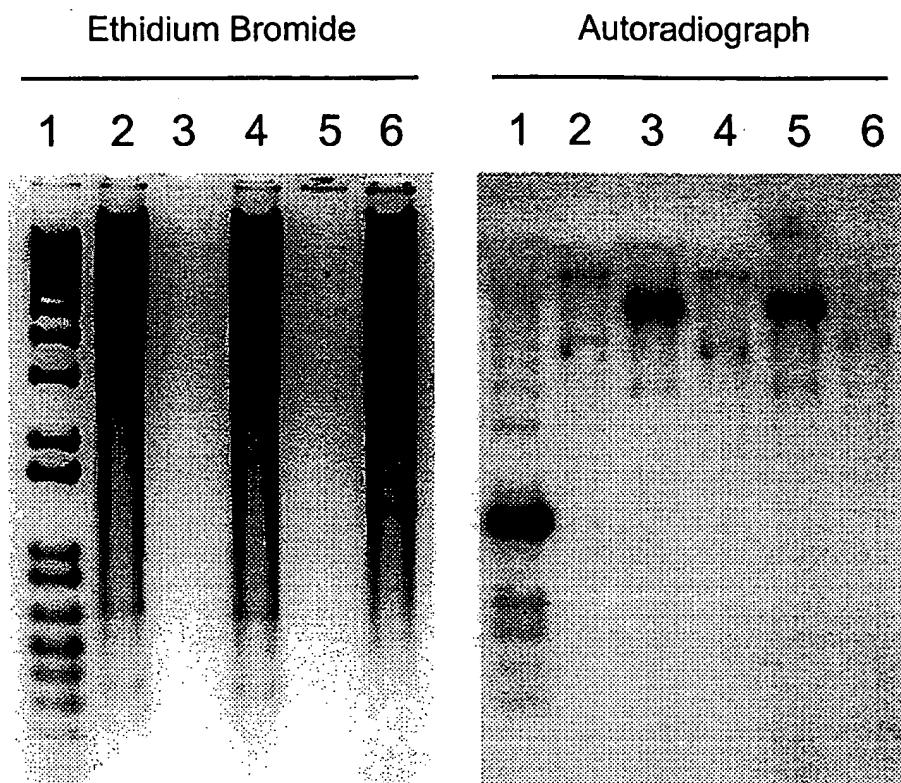
**FIG. 17****GDB Comprehei**

FIG. 18



**FIG. 19**

**FIG. 20****Lanes**

- 1) 1kb ladder
- 2) human female genomic
- 3) 12P11 BAC mus
- 4) human female genomic
- 5) 12P11 BAC mus
- 6) 3T3

## FIG. 1A

11 20 29 38 47 56  
 5' GAG ACT CAC GGT CAA GCT AAG GCG AAG AGT GGG TGG CTG AAG CCA TAC TAT TTT  
 -----  
 65 74 83 92 101 110  
 ATA GAA TTA ATG GAA AGC AGA AAA GAC ATC ACA AAC CAA GAA GAA CTT TGG AAA  
 -----  
 M E S R K D I T N Q E E L W K  
 119 128 137 146 155 164  
 ATG AAG CCT AGG AGA AAT TTA GAA GAA GAC GAT TAT TTG CAT AAG GAC ACG GGA  
 -----  
 M K P R R N L E E D D Y L H K D T G  
 173 182 191 200 209 218  
 GAG ACC AGC ATG CTA AAA AGA CCT GTG CTT TTG CAT TTG CAC CAA ACA GCC CAT  
 -----  
 E T S M L K R P V L L H L H Q T A H  
 227 236 245 254 263 272  
 GCT GAT GAA TTT GAC TGC CCT TCA GAA CTT CAG CAC ACA CAG GAA CTC TTT CCA  
 -----  
 A D E F D C P S E L Q H T Q E L F P  
 281 290 299 308 317 326  
 CAG TGG CAC TTG CCA ATT AAA ATA GCT GCT ATT ATA GCA TCT CTG ACT TTT CTT  
 -----  
 Q W H L P I K I A A I I A S L T F L  
 335 344 353 362 371 380  
 TAC ACT CTT CTG AGG GAA GTA ATT CAC CCT TTA GCA ACT TCC CAT CAA CAA TAT  
 -----  
 Y T L L R E V I H P L A T S H Q Q Y  
 389 398 407 416 425 434  
 TTT TAT AAA ATT CCA ATC CTG GTC ATC AAC AAA GTC TTG CCA ATG GTT TCC ATC  
 -----  
 F Y K I P I L V I N K V L P M V S I  
 443 452 461 470 479 488  
 ACT CTC TTG GCA TTG GTT TAC CTG CCA GGT GTG ATA GCA GCA ATT GTC CAA CTT  
 -----  
 T L L A L V Y L P G V I A A I V Q L  
 497 506 515 524 533 542  
 CAT AAT GGA ACC AAG TAT AAG AAG TTT CCA CAT TGG TTG GAT AAG TGG ATG TTA  
 -----  
 H N G T K Y K K F P H W L D K W M L  
 551 560 569 578 587 596  
 ACA AGA AAG CAG TTT GGG CTT CTC AGT TTC TTT TTT GCT GTA CTG CAT GCA ATT  
 -----  
 T R K Q F G L L S F F A V L H A I  
 605 614 623 632 641 650  
 TAT AGT CTG TCT TAC CCA ATG AGG CGA TCC TAC AGA TAC AAG TTG CTA AAC TGG  
 -----  
 Y S L S Y P M R R S Y R Y K L L N W

659	668	677	686	695	704
GCA TAT CAA CAG GTC CAA CAA AAT AAA GAA GAT	GCC TGG ATT GAG	CAT GAT GTT			
-----	-----	-----	-----	-----	-----
A Y Q Q V Q Q N K E D A W I E H D V					
713	722	731	740	749	758
TGG AGA ATG GAG ATT TAT GTG TCT CTG GGA ATT	GTG GGA TTG GCA ATA CTG GCT				
-----	-----	-----	-----	-----	-----
W R M E I X V S L G I V G L A I L A					
767	776	785	794	803	812
CTG TTG GCT GTG ACA TCT ATT CCA TCT GTG AGT	GAC TCT TTG ACA TGG AGA GAA				
-----	-----	-----	-----	-----	-----
<u>L L A V T S I P S V S D S L T W R E</u>					
821	830	839	848	857	866
TTT CAC TAT ATT CAG AGC AAG CTA GGA ATT GTT	TCC CTT CTA CTG GGC ACA ATA				
-----	-----	-----	-----	-----	-----
F H Y I Q S K L G I V S L L L G T I					
875	884	893	902	911	920
CAC GCA TTG ATT TTT GCC TGG AAT AAG TGG ATA GAT	ATA AAA CAA TTT GTA TGG				
-----	-----	-----	-----	-----	-----
<u>H A L I F A W N K W I D I K Q F V W</u>					
929	938	947	956	965	974
TAT ACA CCT CCA ACT TTT ATG ATA GCT GTT TTC CTT	CCA ATT GTT GTC CTG ATA				
-----	-----	-----	-----	-----	-----
<u>Y T P P T F M I A V F L P I V V L I</u>					
983	992	1001	1010	1019	1028
TTT AAA AGC ATA CTA TTC CTG CCA TGC TTG AGG AAG	AAG ATA CTG AAG ATT AGA				
-----	-----	-----	-----	-----	-----
<u>F K S I L F L P C L R K K I L K I R</u>					
1037	1046	1055	1064	1073	1082
CAT GGT TGG GAA GAC GTC ACC AAA ATT AAC AAA	ACT GAG ATA TGT TCC CAG TTG				
-----	-----	-----	-----	-----	-----
<u>H G W E D V T K I N K T E I C S Q L</u>					
1091	1100	1109	1118	1127	1136
TAG AAT TAC TGT TTA CAC ACA TTT TTG TTC AAT	ATT GAT ATA TTT TAT CAC CAA				
-----	-----	-----	-----	-----	-----
<u>* N Y C L H T F L F N I D I F Y H Q</u>					
1145	1154	1163	1172	1181	1190
CAT TTC AAG TTT GTA TTT GTT AAT AAA ATG ATT ATT	CAA GGA AAA AAA AAA AAA				
-----	-----	-----	-----	-----	-----
<u>H F K F V F V N K M I I Q G K K K K</u>					
AAA AA 3'					
-----					
K					